EVALUATION OF TUMOR INFILTRATING LYMPHOCYTES IN BREAST CANCER

Dissertation submitted in partial fulfillment of the requirements for the degree of

M.D., (PATHOLOGY)

BRANCH-III

INSTITUTE OF PATHOLOGY
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THE TAMIL NADU DR. M.G.R MEDICAL UNIVERSITY

CHENNAI

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CERTIFICATE

This is to certify that this dissertation entitled **"EVALUATION OF TUMOR INFILTRATING LYMPHOCYTES IN BREAST CANCER"** is the original work of **Dr. U. SUGANYA,** in partial fulfilment of the requirement for M.D., (Branch III) in Pathology examination of the Tamilnadu Dr.M.G.R. Medical University to be held in May 2018.

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DECLARATION

I, **Dr. U. SUGANYA,** solemnly declare that the dissertation titled **"EVALUATION OF TUMOR INFILTRATING LYMPHOCYTES IN BREAST CANCER"** is the bonafide work done by me at the Institute of pathology, Madras Medical College under the expert guidance and supervision of **Prof. Dr. SUDHA VENKATESH M.D.,** Professor of Pathology, Institute of pathology, Madras Medical College. The dissertation is submitted to the Tamilnadu Dr. M.G.R Medical University towards partial fulfilment of requirement for the award of M.D., Degree (Branch III) in Pathology.

Place: Chennai

Date: **Dr. U. SUGANYA**

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CERTIFICATE OF APPROVAL

To
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Dear Dr.Suganya.U,

The Institutional Ethics Committee has considered your request and approved your study titled **"EVALUATION OF TUMOR INFILTRATING LYMPHOCYTES (TILs) IN BREAST CANCER " NO. 18102016.**

The following members of Ethics Committee were present in the meeting hold on **04.10.2016** conducted at Madras Medical College, Chennai 3

1.Dr.C.Rajendran, MD.,	:Chairperson
2.Dr.M.K.Muralidharan,MS.,M.Ch.,Dean, MMC,Ch-3	:Deputy Chairperson
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We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary – Ethics Committee

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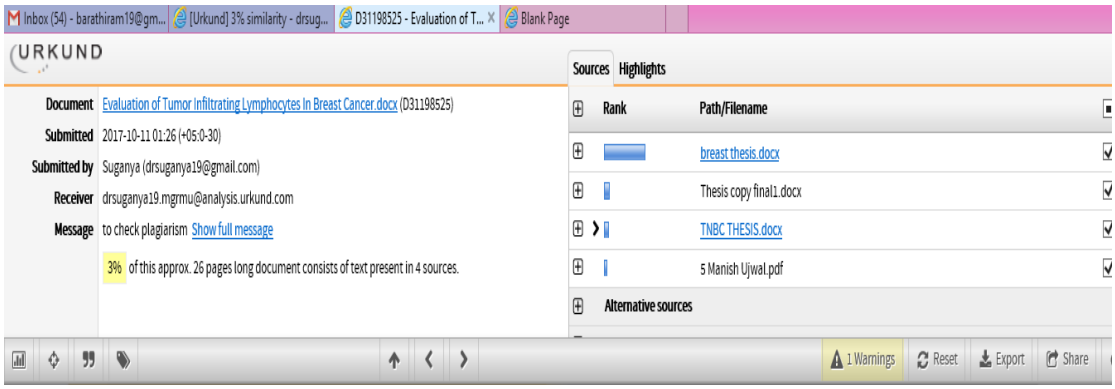


ABBREVIATIONS

ICMR	**:**	**Indian Council of Medical Research**
HRT	**:**	**Hormone Replacement Therapy**
BRCA1	**:**	**Breast Carcinoma 1 Gene**
BRCA2	**:**	**Breast Carcinoma 2 Gene**
ER	**:**	**Estrogen Receptor**
PR	**:**	**Progesterone Receptor**
HER 2 NEU	**:**	**Human Epidermal Growth Factor Receptor 2**
IHC	**:**	**ImmunoHistoChemistry**
P53	**:**	**Protein 53**
mRNA	**:**	**Messenger RiboNucleic Acid**
IDC NOS	**:**	**Infiltrating Ductal Carcinoma Not otherwise Specified**
APC	**:**	**Antigen Presenting Cells**
TILs	**:**	**Tumor Infiltrating Lymphocytes**
TNBC	**:**	**Triple Negative Breast Cancer**
DCIS	**:**	**Ductal Carcinoma Insitu**

CONTENTS

INTRODUCTION

INTRODUCTION

Carcinoma of breast is the most common malignancy in women. It can occur at any age but is uncommon in women less than 25 years and over 80 years. Peak incidence is during 45-60 years.

Inherited mutations account for 10% of carcinomas of breast. The other risk factors include nulliparity, delayed child birth (>30 years), early menarche & over-consumption of alcohol (>3 times per day).

Ductal adenocarcinoma is the commonest type of breast cancer with a relative percentage of >95%. It is further classified into invasive & insitu carcinoma.

The most common histological subtype is Invasive Ductal Adeno Carcinoma –NOS with relative percentage of 75%.

Clinically, it usually presents as a palpable lump in the breast involving the left upper outer quadrant in majority of cases, nipple retraction, peau'd orange appearance and attachment of tumour to underlying chest wall.

Mammographic findings of Invasive Breast Carcinoma shows irregular mass lesion with radiating spikes to uniform & regular edged mass with or without calcification.

Immunohistochemically 40-55% of NST are Luminal A which are ER+VE and HER2/neu –ve. This phenotype is seen in well and moderately differentiated carcinoma, post-menopausal women and it exhibits good

response to hormonal therapy with little response to conventional chemotherapy in a minority of cases.

15-20% of NST are Luminal B which expresses ER & HER2/neu receptors. This phenotype indicates higher grade and higher proliferative index. It is associated with lymph node metastasis but responds well to chemotherapy.

13-25% of NST are basal like which expresses neither ER/ PR nor HER2/neu.

Breast carcinoma is a heterogenous neoplasm with diverse growth rates, different cell clones and metastatic potential. This heterogenous nature of breast carcinoma explains the different clinical behaviour among patients with same pathologic or clinical stage.

Research on “tumor infiltrating lymphocytes in breast cancer” is one of the main field of investigation in recent time.

Several studies have shown that tumor infiltrating lymphocytes is one of the significant prognostic factors in primary breast cancer.Studies have shown that Increasing percentage of tumor infiltrating lymphocytes in breast cancer have better prognosis.

AIMS & OBJECTIVES

AIMS AND OBJECTIVES

- To correlate grade with Tumor Infiltrating Lymphocytes
- To correlate ER, PR, Her2neu status with Tumor Infiltrating Lymphocytes
- To correlate neoadjuvant chemotherapy with Tumor Infiltrating Lymphocytes.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Breast cancer is the most common malignant neoplasm and also the most common cause for cancer deaths in females with more than 1 million cases being newly diagnosed annualy .(1). In United States, around one Lakh new cases are reported every year and approximately 30 thousand women die because of breast carcinoma. The new case reported are high in Northern Europe and North America (91.4 new cases per 100 000 women per year), intermediate in Latin American and southern European countries, low in most of the Asian and African Nations (but rising rapidly in the recent years with increased affluence of some of these countries). In USA, there is a sharp increase in the diagnosis of breast cancer, due to the widespread use of mammography.(2).

According to World Cancer Research Fund International, Breast cancer is the most common cancer in women worldwide with nearly 1.7 million new cases diagnosed in 2012. Breast cancer represents about 12% of all new cancer cases and 25% of all cancers in women.

According to ICMR 2016, while the age adjusted incidence rates of breast cancer in India is lower than western countries, because of the large population the burden of breast cancer is high. Now breast cancer has become the most common female cancer in India.

Breast cancer in India varies from as low as 5 per 100,000 population per year in rural areas to 30 per 100,000 population per year in urban areas.

As most hospital based series report in India gives an incidence of median age of breast cancer patients a decade younger than western countries. The incidence of breast cancer increases with age and this is true with the rest of the world.

Department statistics

In the Institute of pathology, Madras Medical College, the total pathological specimens received in the year 2015 was 11402. Among that, the total number of breast disease specimens was 844, including 380 Breast cancer specimens.

RISK FACTORS

Family History :

Females with 1º relative with breast cancer have a risk of 2-3 times that of the general population, and if the relative was affected at an early age the risk is further increased.(3).

Reproductive history:

Increased risk is seen with early menarche,nulliparity, late age at first birth, and late menopause.(4,5) Breast cancer is rare in those who have undergone oophorectomy before the age of 35 years and reduces the risk to one-third. Women who have their first child before the age of 18 years have only 1/3rd the risk of those whose first child is delayed until age 30.(6).

A reduction in the risk of breast carcinoma among premenopausal women who have lactated has been documented, but no such effect was detected among postmenopausal women with breast cancer.(7). Breast carcinoma risk is increased in postmenopausal women with a increased androgen in plasma .(8).

Fibrocystic disease and epithelial hyperplasia.

These changes in the breast have an increased risk of invasive carcinoma. In some older series, there has been an 2.5-fold overall risk increased whereas in others 2 to 9-fold increased risk was observed only in patients with a previous diagnosis of fibrocystic disease.

Exogenous estrogens.

More recently, a large cohort study and a large case-control study have provided strong evidence for a greater increase risk in women using hormone replacement therapy (HRT) than in those using estrogens alone.(9,10). Very recently, studies have added that recent long-term use of hormone replacement therapy is associated with an increased risk of breast carcinoma, particularly of the lobular type.(11). In December 2002, the hormone estrogen was declared a known human carcinogen by the National Toxicology Program.

Contraceptive agents.

The various epidemiologic studies have shown no increased risk, or at most a very low increase among young long-term users.(12).

Ionizing radiation.

An increased risk of breast carcinoma has been documented with exposure to ionizing radiation, particularly if this exposure occurred at the time of breast development. For example, those who have received irradiation to the mediastinum for Hodgkin lymphoma at early age.(13).

Breast augmentation.

Breast carcinomas are sometimes detected in women who have undergone augmentation mammoplasty.(14). However, the re-analysis of a previously published studies has shown that the incidence of breast carcinoma was neither higher nor lower than that among the general population.(15).

Others.

An interesting association between breast carcinoma and Meningioma is noted.(16).Even more peculiar is that fact that the breast carcinoma may be found to metastasize within the Meningioma. Ataxia–telangiectasia syndrome and Cowden syndrome have an increased risk of breast cancer.(17).

Genetic predisposition

Approximately 5 to 10% of all breast cancers are familial.(18). There are 2 high-penetrance susceptibility genes, when affected by germline mutations, are associated with an increased life-time risk of occurrence of breast cancer as well as few other cancers like ovarian carcinoma identified. (19).

They are BRCA1, located on chromosome 17q21, and BRCA2, sited on 13q12.3 chromosome . (20).

Mutations of this gene are present in around 2% of Ashkenazi Jews; it has been calculated that among carriers the risk for breast carcinoma is 70 to 80% by the age of seventy years. (21).

Study of the breast carcinomas occurring in carriers of BRCA1 mutations has found a increased percentage of carcinomas with features of medullary carcinoma, i.e., carcinomas are of higher grade, mitotically very active, pushing margins, with a syncytial growth pattern, confluent necrosis, negativity for hormone receptors , HER2neu (‘triple negative’), basal-like gene expression profile and with TP53 mutation. (22).

BRCA2-associated cancers are a heterogeneous group without a specific morphological feature or phenotype and mostly positive for ER, PR(hormone receptors).(23).

Other known susceptibility genes account for less than 10% of hereditary breast cancers. The tumor suppressor genes like LKBI/STK11 (Peutz-Jeghers syndrome), PTEN (Cowden syndrome), ATM (ataxia telangiectasia), are seem to be mutated in lesser than 1% of all breast carcinomas.

SPORADIC BREAST CANCER

It is well established that the risk factors for sporadic breast cancers are related to hormone exposure, sex, age at menarche and age at menopause, exogenous estrogens.

Location

About half of the breast cancers are located in the upper outer quandrant, fifteen percent are in the upper medial quadrant, ten percent are in the lower lateral quadrant, seventeen percent are in the central region , five percent are in the lower inner quadrant and three percent breast cancers are diffuse (massive or multifocal).

Several studies have documented the peculiar fact that breast carcinoma is slightly more frequent in the left breast than in the right. In one recent series, the excess for the left side was 13%. (24).

Multicentricity

Fisher et al ,Definition of multicentricity is the presence of tumor in a breast quadrant other than the quandrant containing dominant mass.(25).

Multicentricity is more commonly seen in invasive lobular carcinomas than in invasive ductal carcinomas. A recent study states that multicentric cancers are associated with a lower survival rate than unicentric cancers of the same aggregate volume.

The chance that a woman with invasive breast cancer in one side to develop carcinoma in the contralateral breast is around 5 times that of the general population, and is even greater if a positive family history of breast carcinoma is present. In cases of lobular carcinoma, the number can be as high as 25–50%.

Carcinogenesis and Tumor Progression

The Cell of origin is resident breast tissue stem cells. Most common driver mutations involve the protooncogenes PIK3CA, MYC, HER2, and CCND1 and the tumor suppressor genes TP53, in familial breast cancers (BRCA1 & BRCA2). Once the process is initiated in such cells with a driver mutation, there appear 3 major genetic pathways of carcinogenesis.

The ER positive, HER-2 negative cancers arise via dominant pathway of breast cancer development constituting 50-65% of cases. This subtype is the most common breast cancer subtype in BRCA2 germline mutation.

ER POSITIVE cancers are called as LUMINAL as these cancers closely resemble normal breast luminal epithelail cells in terms of mRNA expression which is dominated by genes that are regulated by estrogen.

Depending upon the proliferation rate (Ki 67) and the response to therapy LUMINAL cancers are subdivided into LUMINAL A and LUMINAL B. Putative precursor lesions of this subtype of breast carcinoma is atypical ductal hyperplasia and flat epithelial atypia.

The HER-2 positive cancers arise through the pathway which is strongly associated with amplifications of HER-2 gene and chromosome number 17q. They constitute around 20 percent of all breast cancers and may either be ER positive or negative. This subtype is the most common breast cancer in p53 germ line mutation (Li-Fraumeni syndrome). Putative precursor lesion of this subtype is termed as atypical apocrine adenosis .

ER negative and HER-2 negative cancers arise through a distinct pathway which is independent of ER mediated changes in the gene expression and HER-2 gene amplification. Precursor lesion of this subtype is yet to be described and so this is the least understood pathway. These cancers comprise about 15% of overall breast cancers, mostly observed in BRCA1 mutation. Sporadic type often has a loss of functional mutations in TP53. These cancers have a “basal-like” pattern of mRNA expression that includes many genes which are expressed in normal myoepithelial cells.

Neoplastic epithelial cells do not develop in isolation and are dependent on interactions with the stromal cells in local microenvironment.

The transition of carcinoma in situ to invasive carcinoma is the final step in carcinogenesis. The molecular events that occur in the normal

formation of new ductal branch points , lobules during puberty and pregnancy, abrogation of the basement membrane, escape from growth inhibition, new blood vessel formation,stromal invasion may be seen in the progression of carcinogenesis. The inflammatory and “wound healing like” tissue reactions that occur during the remodeling of the breast explain the transient increase in breast cancers incidence during and soon after pregnancy, because these changes can facilitate the carcinoma insitu to transform into invasive carcinoma.(26).

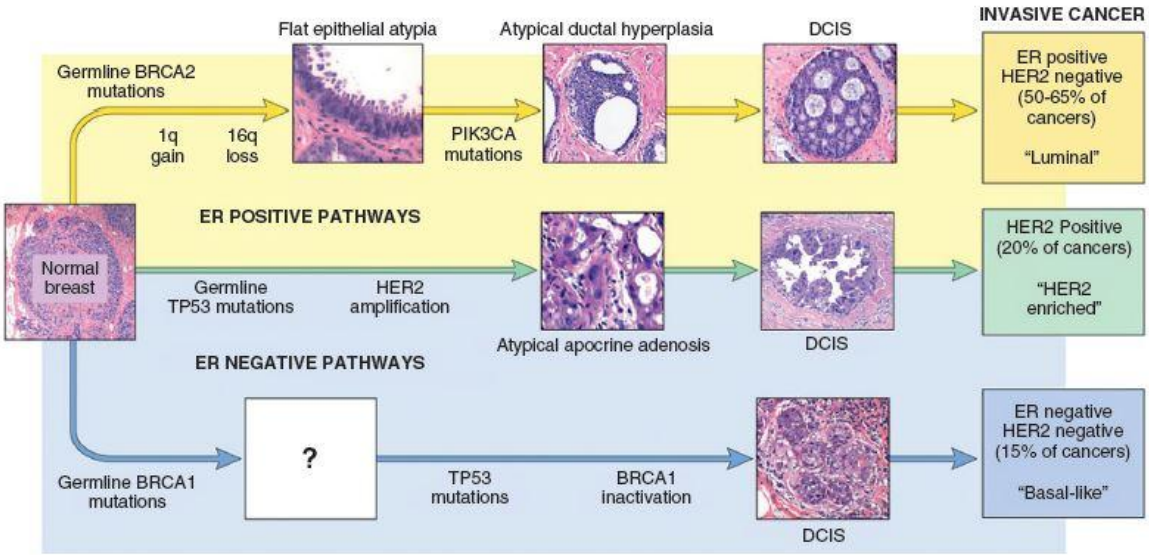


Figure 1: PATHWAYS OF BREAST CANCER DEVELOPMENT

CLINICAL EXAMINATION

- Triple assessment test is done for screening of breast diseases which includes clinical examination, imaging and tissue sampling.

PALPATION

- Clinical examination by palpation remains the extremely useful and considered as one of the best mode for diagnosis of breast carcinoma.

RADIOLOGICAL IMAGING

1. Mammogram:

- The widespread use of mammography made a dramatic change in the diagnosis of breast neoplasm.
- Mammographic screening used for detecting small non palpable carcinoma that were asymptomatic.
- Calcification and density are the primary signs of mammographically detected carcinomas include density

Mammographic Density

- Invasive carcinoma, fibroadenoma or cyst produce mammographic density.
- Most tumors are radiologically dense when compared to the adjacent normal breast parenchyma.

Calcification

- Areas of necrosis, hyalinised stroma or secretion shows calcification.
- Calcification is seen in benign tumors like Hyalinised fibroadenomas,apocrine cysts and sclerosing adenosis.
- Calcification in malignancy are usually tiny, numerous, irregular and clustered.
- Linear branching pattern of calcific deposits are seen in DCIS. DCIS is most frequently detected as calcification in mammogram.
- Infiltrating Ductal adenocarcinomas when small present rarely with calcification unaccompanied by mammographic radiodensity. Lymph node metastasis is rare in such cases.

USG

- Helps in distinguishing between solid and cystic lesions.
- Margins can be delineated in case of solid lesions

MRI

- Because of increased vascularity of the mass , breast carcinomas are detected by uptake of contrast agents .
- Helps in screening the high risk women and those with dense breast .
- Cases of breast implants with rupture are evaluated.

TISSUE SAMPLING METHODS

- Core needle Biopsy
- Excision Biopsy (lumpectomy)
- Incision Biopsy
- Radical and Modified Radical Mastectomy
- Fine needle aspiration cytology

CLASSIFICATION OF BREAST CANCER

Carcinoma of the breast arises from the ductal epithelium in 90% cases while the remaining 10% originate from the lobular epithelium. For variable period of time, the tumour cells remain confined within the ducts or lobules (non-invasive carcinoma) before they invade the breast stroma (invasive carcinoma).

NON-INVASIVE (IN SITU) BREAST CARCINOMA

In general, two types of non-invasive or in situ carcinoma -ductal carcinoma in situ and lobular carcinoma in situ, are characterised histologically by presence of tumour cells within the ducts or lobules respectively without evidence of invasion.

Ductal Carcinoma in situ

Carcinoma in situ confined within the larger mammary ducts is called ductal carcinoma in situ.

The tumour initially begins with atypical hyperplasia of ductal epithelium followed by filling of the duct with tumour cells.

Clinically, it produces a palpable mass in 30-75% of cases and presence of nipple discharge in about 30% patients.

Approximately a quarter of patients of intraductal carcinoma treated with excisional biopsy alone develop ipsilateral invasive carcinoma during a follow-up period of 10 years while the chance of a contralateral breast cancer developing in patients with intraductal carcinoma is far less than that associated with in situ lobular carcinoma.

Lobular Carcinoma in Situ

Lobular carcinoma in situ is not a palpable or grossly visible tumour. Patients of in situ lobular carcinoma treated with excisional biopsy alone develop invasive cancer of the ipsilateral breast in about 25% cases in 10 years as in intraductal carcinoma but, in addition, have a much higher incidence of developing a contralateral breast cancer (30%).

INVASIVE BREAST CARCINOMA

Infiltrating Ductal Carcinoma-NST

Infiltrating ductal carcinoma-NST is the classic breast cancer and is the most common histologic pattern accounting for 80% cases of breast cancer.

Clinically, majority of infiltrating ductal carcinomas have a hard consistency due to dense collagenous stroma (scirrhous carcinoma).

Retraction of the nipple and attachment of the tumour to underlying chest wall may be present.

Grossly, the tumour is irregular, 1-5 cm in diameter, hard cartilage-like mass that cuts with a grating sensation. The cut surface of the tumour is grey-white to yellowish with chalky streaks and often extends irregularly into the surrounding fat.

Microscopically**,** as the name NST suggests, the tumour is different from other special types in lacking a regular and uniform pattern throughout the lesion. A variety of histologic features commonly present are as follows,

i) Anaplastic tumour cells forming solid nests, cords, poorly-formed glandular structures and some intraductal foci.

ii) Infiltration by these patterns of tumour cells into diffuse fibrous stroma and fat.

iii) Invasion into perivascular and perineural spaces as well as lymphatic and vascular invasion.

Infiltrating (Invasive) Lobular Carcinoma

Invasive lobular carcinoma comprises about 10% of all breast cancers. This peculiar morphologic form differs from other invasive cancers in being more frequently bilateral; and within the same breast, it may have multicentric origin.

Grossly, the appearance varies from a well-defined scirrhous mass to a poorly defined area of induration that may remain undetected by inspection as well as on palpation.

Microscopically , there are 2 distinct features :

- A characteristic single file (Indian file) linear arrangement of stromal infiltration by the tumour cells with very little tendency to gland formation is seen. Infiltrating cells may be arranged concentrically around ducts in a target-like pattern.
- Individual tumour cells resemble cells of in situ lobular carcinoma. They are round and regular with very little pleomorphism and infrequent mitoses. Some tumours may show signet-ring cells distended with cytoplasmic mucin.

Tubular Carcinoma

Tubular carcinoma comprises about 6% cases of invasive ductal carcinoma and has more favourable prognosis. These tumours are generally small (~1 cm diameter) ill-defined and gritty nodules.

Histologically, the tumour is highly well-differentiated having following characteristics:

- The tumour is almost exclusively composed of tubules having angulated shape.
- The tumour cells are regular and form a single layer in well-defined tubules in a fibrous stroma

Medullary Carcinoma

Medullary carcinoma is a variant of ductal carcinoma and comprises about 2% of all breast cancers. The tumour has a significantly better prognosis ,probably due to good host immune response in the form of lymphoid infiltrate in the tumour stroma.

Grossly, the tumour is characterised by a large, well-circumscribed, rounded mass that is typically soft and fleshy or brain-like and hence the alternative name of ‘encephaloid carcinoma’. Cut section shows areas of haemorrhages and necrosis.

Histologically, medullary carcinoma is characterised by 2 distinct features :

- Sheets of large, pleomorphic tumour cells with abundant cytoplasm, large vesicular nuclei and many bizarre and atypical mitoses are diffusely spread in the scanty stroma.
- The loose connective tissue stroma is scanty and usually has a prominent lymphoid component as aggregates and infiltrate.

Colloid (Mucinous) Carcinoma

This pattern of breast cancer is seen in about 2% cases, occurs more frequently in older women and is slow-growing. Colloid carcinoma has better prognosis than the usual infiltrating ductal carcinoma.

Grossly, the tumour is usually a soft and gelatinous mass with well-demarcated borders.

Histologically, colloid carcinoma contains large amount of extracellular epithelial mucin and acini filled with mucin. Cuboidal to tall columnar tumour cells, some showing mucus vacuolation, are seen floating in large lakes of mucin.

Papillary carcinoma

It is a rare variety of infiltrating ductal carcinoma in which the stromal invasion is in the form of papillary structures.

Adenoid cystic carcinoma

Adenoid cystic or invasive cribriform carcinoma is a unique histologic pattern of breast cancer in which there is stromal invasion by islands of cells having characteristic cribriform appearance. The tumour has an excellent prognosis.

Secretory carcinoma

This pattern is found more frequently in children and young girls and has a better prognosis. The tumour is generally circumscribed which on

histologic examination shows abundant intra- and extracellular PAS-positive clear spaces due to secretory activity of tumour cells.

Inflammatory carcinoma

Inflammatory carcinoma of the breast is a clinical entity and does not constitute a histological type. The term has been used for breast cancers in which there is redness, oedema, tenderness and rapid enlargement. Inflammatory carcinoma is associated with extensive invasion of dermal lymphatics and has a dismal prognosis.

Metaplastic carcinoma

Rarely, invasive ductal carcinomas, besides epithelial elements, may have various components of metaplastic alterations such as squamous metaplasia, cartilaginous and osseous metaplasia, or their combinations.

The term metaplastic carcinoma includes various categories such as carcinosarcoma, spindle cell carcinoma, carcinoma with osteoclast-like giant cells and squamous cell carcinoma.

MOLECULAR CLASSIFICATION OF BREAST CANCER: (27,28,29,30,31)

Luminal A

- This phenotype is seen in 40% - 50% of the IDC NOS type of breast carcinoma.

- It includes ER positive and HER2-neu negative tumor.
- Most of these tumors are moderately to well differentiated with increased occurence among post menopausal females.
- The tumors in this subtype respond well to hormonal treatment.

Luminal-B

- This phenotype is seen in 15% to 20% of IDC-NOS type of breast cancer.
- They are triple receptor positive tumors with expression of ER, PR & HER2neu.
- They are of higher grade tumors with increased proliferating potential.
- Increased frequency of metastasis to lymph nodes is seen.
- These tumors respond well to chemotherapy.

Normal Breast Like

- This phenotype accounts for about 6% - 10% of IDC NOS type of breast carcinoma.
- This group consists of well differentiated ER positive & HER2neu negative tumors. They show similar gene expression pattern like that of normal breast tissue.

Basal Like

- This phenotype accounts for 13% to 25% of IDC NOS type tumors.
- This type of breast carcinomas are characterized by the absence of PR, ER & HER2neu expression ,but expressing basal myoepithelial markers like P63, P-Cadherin and of progenitor cells / putative stem cells (CK 5/6)
- This group is referred as “TRIPLE NEGATIVE” carcinomas.
- Medullary & Metaplastic carcinomas come in this category.
- Breast carcinomas harboring BRCA1 mutations belong to this category.
- They are of high grade tumors with increased proliferating potential and aggressive clinical behaviour.
- They are frequently associated with CNS and Visceral metastasis.
- Complete response following chemotherapy is observed in only 15-20% of cases.

HER2neu Positive

- This phenotype is seen in about 7% - 12% of IDC NOS type of breast cancers.
- This group includes carcinomas showing HER2neu over expression and ER / PR negativity.
- The overexpression of HER2neu in more than ninety percent of these cancers is because of the amplification of the DNA segment on

chromosome 17q21 which harbours the HER2neu gene and varying number of adjacent genes.

- They are poorly differentiated tumors generally with increased proliferative potential & associated with increased frequency of CNS metastasis.

PROGNOSTIC FACTORS

In the counseling of the patients regarding the likely outcome of the disease and for the appropriate treatment ,the knowledge about the prognostic factors is important.

AGE OF THE PATIENT

Better prognosis is seen in women less than fifty years of age. Prognosis declines after the age of 50.(32)

SIZE

Size is considered as an important prognostic factor and studies show good correlation between nodal status and survival rate.(33,34). For the definition of minimal breast carcinoma size is one of the two criteria, which includes all insitu carcinomas regardless of size and the invasive carcinomas of <1cm in diameter.

SITE

Tumors located in the upper inner and lower inner quadrants have greater risk of (50%) relapse and tumor related death than the laterally located tumors.(35)

CYTOARCHITECTURAL TYPE

There is no prognostically significant difference between ordinary infiltrating ductal and lobular carcinoma.(36). Morphological variants like Mucinous, Medullary, Papillary, Tubular ,Cribriform ,secretory and Adenoid cystic carcinoma have good prognosis.

Variants like Metaplastic, Squamous cell carcinoma, Neuroendocrine, Inflammatory and Signet ring cell carcinoma are aggressive tumors having bad prognosis.(37).

PRESENCE OR ABSENCE OF INVASIVENESS

In carcinomas of ductal type that have both in situ and invasive component, a significant relationship exists between the proportion of the invasive component and the probability of metastasis to lymph nodes.

The amount of insitu component correlates with incidence of multicentricity and indirectly with probability of occult invasion.(38).

Insitu ductal malignancies of the comedocarcinoma type can also be associated with metastases in the absence of a detectable invasion.

TUMOR NECROSIS

Tumor necrosis is associated with reduced survival rates and increased nodal metastases , particularly if it is very extensive.(39). This feature is usually associated with tumors having high histologic grade.(40).

TYPE OF MARGINS

Tumors with infiltrating margins have a worse prognosis than the tumors with pushing margins.(41).

MICROSCOPIC GRADE

Grading is done based on Nottingham Modification of Bloom Richardson system. (42).Ellis et al established that there is an excellent correlation between the Nottingham grading system and patient's survival rate and metastasis.

SKIN INVASION

Breast carcinomas with overlying skin infiltration are associated with decreased survival rate.(43)

NIPPLE INVASION

Carcinomas involving the nipple areolar complex is associated with higher incidence of axillary metastasis.(44).

BLOOD VESSEL EMBOLI

Vascular emboli shows a high association with histological grade, size of the tumor, tumor type, lymph node status and distant metastasis. Tumors with vascular invasion is associated with poor prognosis.(45).

LYMPHATIC TUMOUR EMBOLI

There is increased risk of tumor recurrence if lymphovascular invasion is present.(46).

LYMPH NODE STATUS

Metastatic deposit in the axillary lymph nodes is considered as a poor prognostic factor. Number of nodes involved, level of the nodes and amount of tumor cells present in the node, presence or absence of the tumor cells in the efferent blood vessels have an important implication in the patient's survival.(47)

BRCA-1 STATUS

The carcinomas developing in BRCA 1 mutation carriers are associated with overall poor survival rate, if they have not received adjuvant chemotherapy.(48). Absent (or) reduced nuclear BRCA 1 expression measured using immunohistochemistry is associated with many microscopic unfavorable features and also shorter disease free intervals, whereas cytoplasmic expression of this specific marker is associated with the development of tumor recurrence.(49).

STAGING (TNM) (Annexure II)

PROLIFERATION RATE

The proliferation rate is measured with mitotic counts, IHC detection of cellular proteins like Ki67, Cyclins, and flow cytometry. Poor prognosis is observed in tumors with high proliferation rate but the response to chemotherapy is better. It is also be measured by S-Phase fraction (SPF) and with thymidine labeling index.(50).

OTHER PROGNOSTIC FACTORS

Many factors like lymphocytic infiltration, Tumor necrosis, Skin involvement, association with pregnancy and lactation, keratin, BRCA mutation and vimentin expression also have variable prognostic implications in breast cancer.

TUMOR IMMUNITY AND LYMPHOCYTES IN CANCER:

In cancer, neoplastic transformation alters the structure of tissues and induces immune responses leading to the elimination of developing tumors. However, incomplete elimination of transformed cells results in escape from immune control. This process is known as cancer immunoediting and is supported by a large body of experimental data and clinical evidence showing that the intact immune system can prevent and control cancer through the generation of effective tumor-specific immune responses [51].

Immunoediting describes the process of malignant progression on the basis of tumor and immune cell interactions in three phases:

(1) elimination, where cancerous cells are eliminated following immunosurveillance;

(2) equilibrium, where transformed cells are held in control but are not eliminated by the immune system; and

(3) escape, where tumor cell modifications shape disease progression

Proinflammatory type I immunity, which includes CD4 and CD8 T-helper lymphocytes, is the immune response needed to eliminate cancer.[52]

The ability to suppress immunity is crucial to protect normal tissues from collateral damage during immune responses against any type of pathogen. The cells of both the innate (neutrophils, monocytes, macrophages, and a host of APC) and the adaptive (B and T lymphocytes) immune system work together to respond to any type of pathogens, including tumor antigens.[52]

Innate immune cells are required by B and T cells in order for them to identify immunogenic proteins, thus the subsequent generation of adaptive immunity allows for the development of memory cells, which are lymphocytes that remain in lymph nodes. Most antigens present in breast cancer are self-proteins that can stimulate T cells and also induce a regulatory immune response.[52]

Breast cancer is capable of stimulating the immune system. Furthermore, the intensity of tumoral immune response influences the

effectiveness of cancer therapy, and is correlated with favorable clinical outcome in this disease.

Some breast tumors have substantial lymphocytic infiltration, and tumor-infiltrating lymphocytes (TILs) have been recently proposed as a surrogate marker of adaptive immune response.

The interaction of the immune system with tumor cells in breast cancer appears to be associated with triple negative breast cancer (TNBC) and HER2-positive breast cancer, and they are thought to be more immunogenic than luminal A carcinomas.

There is an emerging concept that the response to chemotherapy is at least partly dependent on an immunological reaction against tumor cells that are dying during the chemotherapy. The large genetic and epigenetic changes present in most cancer cells provide many tumor-associated-antigens that the immune host system can recognize, thereby requiring tumors to develop specific immune resistance mechanisms. An important immune resistance mechanism involves immune check-points, which normally mediate immune tolerance and mitigate collateral tissue damage.[53]

Infiltration of immune cells, particularly infiltration of anti-tumor type 1 lymphocytes, has predicted improved prognosis in many different tumor types including colon, ovarian, lung and breast cancer . Historically breast cancer was not thought to be immunologically active, particularly when compared to tumors such as melanoma.[54]

Page`s et al given the strong evidence for the usefulness of the combined evaluation of memory and cytotoxicity for the prediction of tumor recurrence and survival in patients with stages I and II colorectal cancer.[55].

Meta analysis by Hwang demonstrate that a lack of intraepithelial TILs is significantly associated with a worse survival among patients and Intraepithelial TILs are a robust predictor of outcome in ovarian cancer.[56].

Over the recent years, new results from multiple groups have pointed towards Tumor Infiltrating Lymphocytes (TILs) as prognostic and predictive biomarkers in breast cancer and this is not a new concept.

For instance, it has been known since 1940s that a subtype of breast cancer that characteristically exhibits a very large proportion of stromal lymphocytic infiltrate, called medullary carcinoma, has been associated with excellent prognosis after aggressive local therapy in spite of high histologic grade and axillary lymph node metastases [57].

Tumor-infiltrating lymphocytes are white blood cells that have left the bloodstream and migrated into a tumor. They are mononuclear immune cells, a mix of different types of cells (i.e., T cells, B cells, NK cells, macrophages) in variable proportions, T cells being the most abundant cells. [58]They can often be found in the stroma and within the tumour itself.

TILs are implicated in killing tumor cells. The presence of lymphocytes in tumors is often associated with better clinical outcomes.[59-61]

While patients are most frequently diagnosed in the escape phase, this relationship between the tumor and host immunity continues to evolve and sometimes with it the magnitude of the antitumor immune response.

Even at advanced disease stages, immune parameters have now been recognized as directly or indirectly influencing patient survival . Recently, new therapies that reactivate anticancer immune responses to cancer, for example in melanomas and lung cancer, have entered clinical practice and have improved outcome [62,63].

In the elimination phase, the innate and adaptive immune system coordinate to detect and destroy cancer cells before clinical presentation. At this stage the balance is towards antitumor immunity stimulated by natural killer (NK) cells, T cells, and increased pro-immune factors in the tumor microenvironment [64]. In equilibrium, there is a balance between antitumor and tumor-promoting factors, thus maintaining the tumor in a functionally dormant state [64]. Well-documented escape mechanisms of breast cancer cells include decreased immune recognition through reduced expression of major histocompatibility complex class I (MHC I) and/or co-stimulatory molecules and increased expression of immunosuppressive factors. This results in reduced clearance (lysis) via CD8+ cytotoxic T lymphocytes (CTLs) [65].

The adaptive immune response to breast cancer can be seen in infiltrating breast lesions as early as benign breast atypia and increases in density as invasive malignancy develops. In one retrospective study of 53

mastectomy samples, increased B-cell and T-cell immune infiltrate was identified in benign ductal hyperplasia, increased in ductal carcinoma in situ (DCIS), and was found in the greatest magnitude in invasive breast cancer .[66]

The presence of both B and T lymphocytes in the neoplastic lesions of the breast is in agreement with previous reports, and suggests the participation of both cell-mediated and humoral immunity in mammary carcinogenesis. Most of the mononuclear inflammatory cell infiltrate was composed of CD3+ T cells.[67]

Cancers other than breast cancer also show the association between an abundance of TILs and the de-differentiation status of tumor cells, including lymphoepithelial carcinoma of the nasopharynx, [68] stomach cancer (gastric carcinoma with lymphoid stroma),colon cancer (medullary carcinoma),[69] and lung cancer (lymphoepithelioma-like carcinoma).[70] High-grade renal cell carcinoma was associated with more pronounced infiltration by CD8 + T cells.[71] Considering this, the correlation between the de-differentiation status and prominence of TILs is likely to be common in various types of cancer.

Methodological Recommendations For Evaluating TILs In Breast Cancer (72)

The recommendations of TIL working group 2014 asked the participants with experience in evaluating TILs to complete a questionnaire covering topics pertinent to their assessment in breast cancer. The goal of this approach was to derive a consensus based on current experience within the group and use it as

the foundation for this guideline. Based on these discussions, the working group participants made recommendations for harmonizing TILs evaluation.

Technical Issues For Evaluation Of TILS In Breast Cancer

1) Microscope magnification does not really make a difference, but usually a magnification of ×200–400 (ocular ×10, with an objective of ×20–×40) is recommended.
2) Slide thickness is not critical, with a standard thickness of 4–5 μm considered optimal. The majority of existing experience is based on scoring 4–5 μm sections of formalin fixed and paraffin embedded (FFPE) tissues, while the feasibility of TILs evaluation on frozen sections is undocumented outside of a research setting and thus cannot be recommended for routine use at the present time.
3) TILs can be evaluated using core biopsies in the neoadjuvant setting as well as surgical specimens in the adjuvant setting.
4) Tissue microarrays (TMAs) were not recommended for evaluating TILs, since there was no published evidence that TMAs mirror the potential heterogeneity of TILs, and the number of cores needed and the defined core diameter, accurately reflecting TIL composition in a full section are unknown.
5) The lymphocytes should be scored (granulocytes and other polymorphonuclear leukocytes are excluded). The quantitative assessment of other mononuclear cells such as dendritic cells and macrophages is currently not recommended, although there is increasing

evidence that they may be functionally important since they are observed in TLS.

6) Several studies used immunohistochemistry to assess the clinical importance of subtyping lymphocytes. CD45, CD8, CD3 and various other markers expressed on lymphoid cells have been tested and while immunohistochemistry may improve accuracy, but there is no added value. The TILs working group does not currently recommend the immunohistochemistry.
7) Machine scoring approaches have not been published in large series with consistent methodology.
8) It is unknown if either RNA or protein classification of TILs by will reveal prognostic and predictive value beyond that achievable by simple morphology. New techniques, like CyTOF , can review protein-based signatures of inflammatory infiltrate. Clinical utility will drive the development of specific immune markers

RECOMMENDATIONS FOR ASSESSING TUMOR-INFILTRATING LYMPHOCYTES (TILS) IN BREAST CANCER

1) TILs should be reported for the stromal compartment (=% stromal TILs). The denominator used to determine the % stromal TILs is the area of stromal tissue (i.e. area occupied by mononuclear inflammatory cells over total intratumoral stromal area), not the number of stromal cells (i.e. fraction of total stromal nuclei that represent mononuclear inflammatory cell nuclei).

2) TILs should be evaluated within the borders of the invasive tumor.
3) Exclude TILs outside of the tumor border and around DCIS and normal lobules.
4) Exclude TILs in tumor zones with crush artifacts, necrosis, regressive hyalinization as well as in the previous core biopsy site.
5) All mononuclear cells (including lymphocytes and plasma cells) should be scored, but polymorphonuclear leukocytes are excluded.
6) One section (4–5 μm, magnification ×200–400) per patient is currently considered to be sufficient.
7) Full sections are preferred over biopsies whenever possible. Cores can be used in the pretherapeutic neoadjuvant setting; currently no validated methodology has been developed to score TILs after neoadjuvant treatment.
8) A full assessment of average TILs in the tumor area by the pathologist should be used. Do not focus on hotspots.
9) The working group's consensus is that TILs may provide more biological relevant information when scored as a continuous variable, since this will allow more accurate statistical analyses, which can later be categorized around different thresholds. However, in daily practice, most pathologists will rarely report for example 13.5% and will round up to the nearest 5%–10%, in this example thus 15%.
10) TILs should be assessed as a continuous parameter. The percentage of stromal TILs is a semiquantitative parameter for this assessment, for

example, 80% stromal TILs means that 80% of the stromal area shows a dense mononuclear infiltrate. For assessment of percentage values, the dissociated growth pattern of lymphocytes needs to be taken into account. Lymphocytes typically do not form solid cellular aggregates; therefore, the designation ‘100% stromal TILs’ would still allow some empty tissue space between the individual lymphocytes.

11) No formal recommendation for a clinically relevant TIL threshold(s) can be given at this stage. The consensus was that a valid methodology is currently more important than issues of thresholds for clinical use, which will be determined once a solid methodology is in place. Lymphocyte predominant breast cancer can be used as a descriptive term for tumors that contain ‘more lymphocytes than tumor cells’.

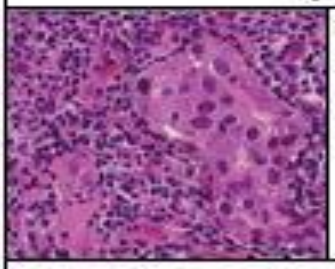
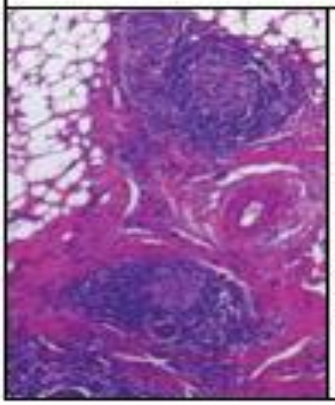
Morphology	Definition and biological relevance	Diagnostic relevance
Lymphocyte-predominant breast cancer (LPBC)		
	Working category to describe tumors with "more lymphocytes than tumor cells".	Definitions vary across studies with stromal TILs of 50–60% used as a threshold. LPBC can be used for predefined subgroup analyses and for description of tumors with a particularly high immune infiltrate, however, keep in mind that TILs are a continuous parameter and the threshold for LPBC is still arbitrary.
Stromal TILs		
	Indicator of increased accumulation of immune-cells in tumor tissue	Stromal TILs have been shown to be predictive for increased response to neoadjuvant chemotherapy as well as improved outcome after adjuvant chemotherapy. Based on current data, this parameter is the best parameter for characterization of TILs.
Intratumoral TILs		
	TILs with direct cell-cell contact with carcinoma cells, might be an indicator of direct cell-based anti-tumor effects.	Several studies have shown that intratumoral TILs and more difficult to evaluate and do not provide additional predictive/prognostic information compared to stromal TILs.
TILs at the invasive margin		
The localization of TILs are the invasive edge is included in the evaluation approach presented in this guideline.		For breast cancer there are no studies with a separate evaluation of TILs at the invasive edge. For practical purposes, the reliable evaluation of the invasive edge might be difficult when using core biopsies in the neoadjuvant setting.
Tertiary lymphoid structures (TLS)		
	Typically localized in the surrounding area of the tumor, TLS might be localized in normal tissue directly adjacent to the tumor, consisting of a T cell zone next to a B cell follicle, often with germinal centers.	While these structures may be important for the biology of tumor-immune reactions, they are not yet optimized for non-research based assessments, The main problem is that TILS have a spatial heterogeneity and are principally localized in areas surrounding the tumor. They might not be in the plane of the tissue section that is being evaluated, in particular when using core biopsies. Furthermore, it might be difficult to distinguish lymphoid aggregates from true TILS, in particular when the germinal center is not in the plane of the section.

Figure 2: Morphology , definitions , biological and diagnostic relevance of the different immune infiltrates found in breast cancer.

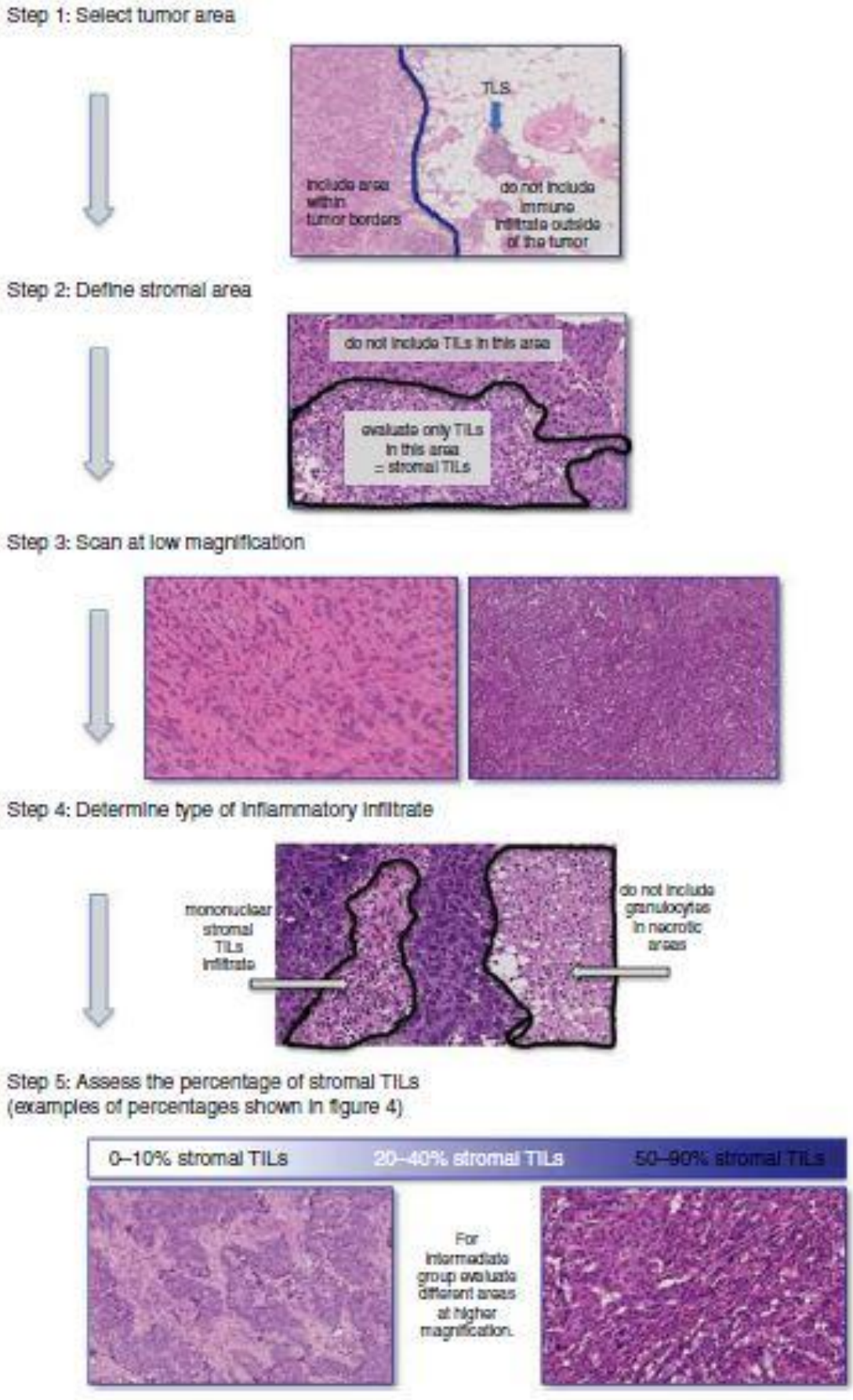


Figure 3 : Standardized approach for TILs evaluation in Breast cancer

MATERIALS & METHODS

MATERIALS AND METHODS

This study is a descriptive retrospective study of Primary breast carcinomas conducted in the Institute of Pathology, Madras Medical College and Rajiv Gandhi Government General hospital, Chennai during the period between January 2013 to December 2016.

SOURCE OF DATA

A total of 2083 breast biopsy specimens were received in our surgical pathology department during this three year period. Out of which 943 were malignant. Of these 325 cases were diagnosed as malignant in mastectomy specimens. The invasive ductal carcinoma NOS and special subtype cases reported in mastectomy specimens received in the Institute of Pathology, Madras Medical College between January 2013 to December 2015 from the Department of General Surgery and surgical Oncology, Madras Medical College and Rajiv Gandhi Government General Hospital was included in this study.

INCLUSION CRITERIA

- Cases diagnosed as primary carcinoma breast in mastectomy specimens

EXCLUSION CRITERIA

- Benign tumors,
- Benign and malignant phyllodes,
- Non neoplastic lesions of breast and
- Small biopsies.

METHOD OF DATA COLLECTION

Of the total cases reported during this study period, ER, PR and HER2neu expression was studied for 188 cases. 52 cases were given neoadjuvant chemotherapy. Detailed history of the cases regarding age, sex, side of the breast, type of procedure, details of gross characteristics such as tumor size, nodal status details were obtained for those 188 cases from surgical pathology records. Formalin fixed tissue were cut, processed and paraffin embedded.

4μm thick sections of the paraffin tissue blocks were cut and stained with eosin and hematoxylin. Slides were collected from slide filing and were reviewed and graded using the Nottingham modification of the Scarff Bloom Richardson Grading system (Annexure III). 93 cases from Invasive ductal carcinoma NOS and 07 cases from special types such as metaplastic, mucinous, medullary, IDC- NST with papillary features were randomly selected from the total cases and their representative formalin fixed paraffin embedded tissue samples were subjected for analysis of Tumor Infiltrating Lymphocytes with H&E slides and with CD 45 And CD 3 expression. Slides were evaluated for percentage of tumor infiltrating lymphocytes. The results were recorded with photographs.

IMMUNOHISTOCHEMICAL EVALUATION

Immuno-histochemical analysis of ER, PR, H2N , CD 45 and CD 3 were done in Paraffin embedded tissue samples using supersensitive polymer HRP system based on non-biotin polymeric technology.

Table 1: Immunohistochemical markers used in the current study

Antigen	Vendor	Clone	Dilution	Positive control
ER	Dako	Rabbit Monoclonal	Ready to use	Breast
PR	Dako	Mouse monoclonal	Ready to use	Breast
HER2neu	Dako	Rabbit Monoclonal	Ready to use	Breast
CD45	Pathnsitu	Rabbit Monoclonal	Ready to use	Lymphnode
CD3	Pathnsitu	Mouse polyclonal	Ready to use	Lymphnode

PREPARATION OF SLIDES:

1. Formalin fixed paraffin embedded tissue samples and cut into 4µ thick sections and transferred to charged slides
2. The slides were incubated overnight at 58ºC .
3. The sections were deparaffinized in 2 changes of xylene for 10 minutes
4. The sections were dehydrated with two changes of absolute alcohol for 10 minutes.
5. The slides were immersed in tap water for 10 minutes.
6. The slides were then kept in 2 changes of distilled water for 2 minutes.

ANTIGEN RETRIEVAL:

7. Antigen retrieval was done with microwave oven with appropriate buffer in appropriate temperature for 25 minutes.
8. The slides were cooled to room temperature and then washed in distilled water for 2 minutes.
9. Apply peroxidase block over the sections for 10 minutes.
10. slides are washed in 2 changes of wash buffer for 5 minutes .

ANTIBODY APPLICATION:

11. The sections were drained (without washing) and appropriate primary antibody was applied over the sections and incubated for 45-60 minutes.
12. The slides were washed twice in wash buffer for 5 minutes each.
13. The slides were covered with Anti – Polyvalent HRP Polymer for 20 minutes.
14. The slides were washed in wash buffer for 5 minutes x 3changes.

CHROMOGEN APPLICATION:

15. 1 drop of DAB chromogen to 1 ml of DAB buffer are mixed to make a DAB subtrate.
16. DAB solution was applied on the sections for 5 minutes.
17. The slides were then washed well with distilled water for 5 minutes.

18. The sections were counterstained with Haematoxylin stain for 2 seconds (1 dip).
19. The slides were then washed in running tap water for 3 minutes.
20. The slides are air dried, cleared with xylene and mounted with DPX.

INTERPRETATION & SCORING SYSTEM

ER and PR

Hormone receptors like Estrogen and Progesterone receptor, when expressed show a nuclear positivity. The number of cells expressing and their intensity of staining is scored as two values and a composite score based on percentage plus intensity of more than 2 is considered to be positive. (Annexure IV).

H2N

HER 2-neu expression is demonstrated in tumor cells as cytoplasmic expression is graded as 1+, 2+ and 3+.(Annexure IV).

TUMOR INFILTRATING LYMPHOCYTES(TILS)

Tumor infiltrating lymphocytes has been evaluated with CD45 and CD3 expression. Only Stromal lymphocytes are taken for evaluation. Percentage of TILs are evaluated and classified as 0-10%, 20-40% and 50-90% as per the evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014.

STATISTICAL ANALYSIS

The Statistical analysis for this study was done using the software IBM Statistical Package for social science (SPSS) version 20. The correlation between Tumor Infiltrating Lymphocytes and different clinic-pathological parameters like age group, size, side, histopathology, grade, lymph node status, Neo adjuvant chemotherapy, Hormonal receptors like ER, PR and HER2neu was made and strength of association was calculated by Pearson Chi square test and P value less than 0.05 are considered statistically significant.

OBSERVATIONS & RESULTS

OBSERVATION AND RESULTS

In a study period of three years from January 2013 to December 2015 a total of 2083 breast specimens were received in Institute of Pathology, Madras Medical College for histological examination. Among which the malignant breast tumors are total 943 cases, benign breast tumors are 926 cases and non-neoplastic lesions are 214 cases.

Among the 100 breast specimens, all were diagnosed malignant following modified radical mastectomy and all were evaluated for steroid hormone receptor and Her2neu expression. Among the 100 cases, 52 were given neo-adjuvant chemotherapy following which modified Radical Mastectomy was done.

Table 2 : Age Wise Distribution Of Carcinoma Breast

Age Range	Frequency	Percent
21-30	1	1.0
31-40	22	22.0
41-50	34	34.0
51-60	30	30.0
61-70	12	12.0
71-80	1	1.0
Total	100	100.0

The highest incidence of breast cancers was found in the age group of 41-50 years. The median age of the patient in this study was 50. The youngest age of presentation of breast cancer was 26 years in this study. 57 % of patients are below the age group of 50 %.

Figure 4: Age Wise Distribution Of Carcinoma Breast

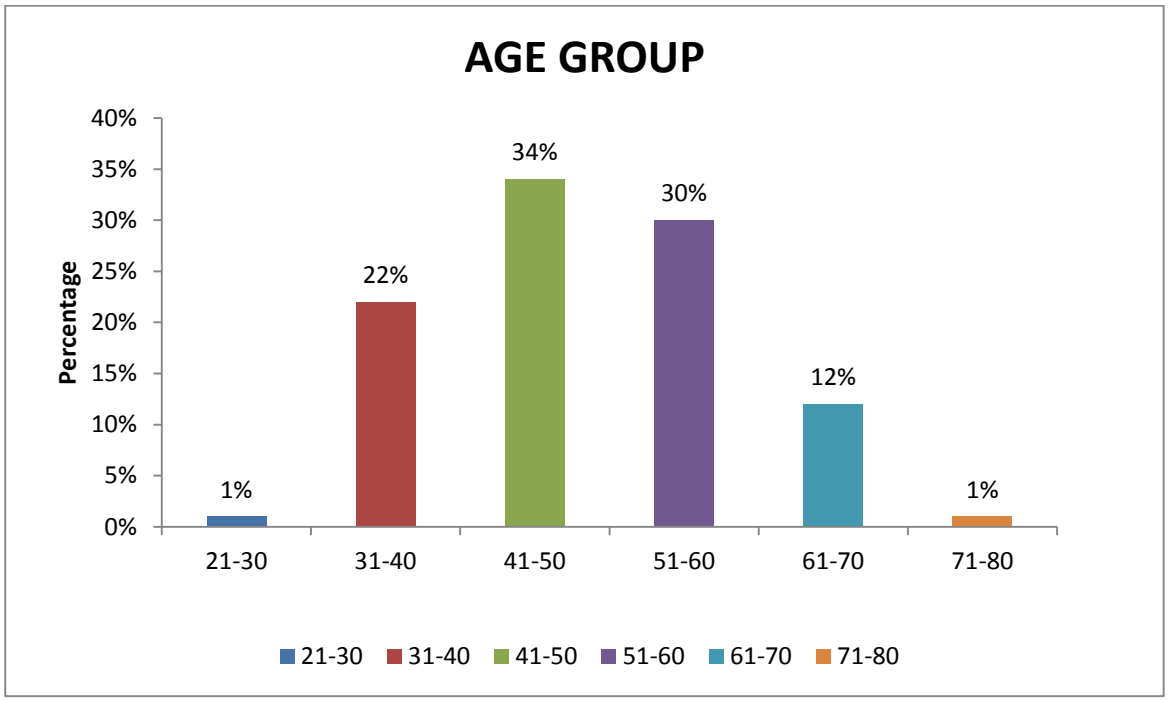


Table 3: Distribution Of Side In Primary Carcinoma Breast

Side Of The Tumor	Frequency	Percent
Left	60	60.0
Right	40	40.0
Total	100	100.0

In our study 60 cases (60%) involved the left Breast and 40 cases (40%) involved the right side of breast.

Figure 5: Distribution Of Side In Primary Carcinoma Breast

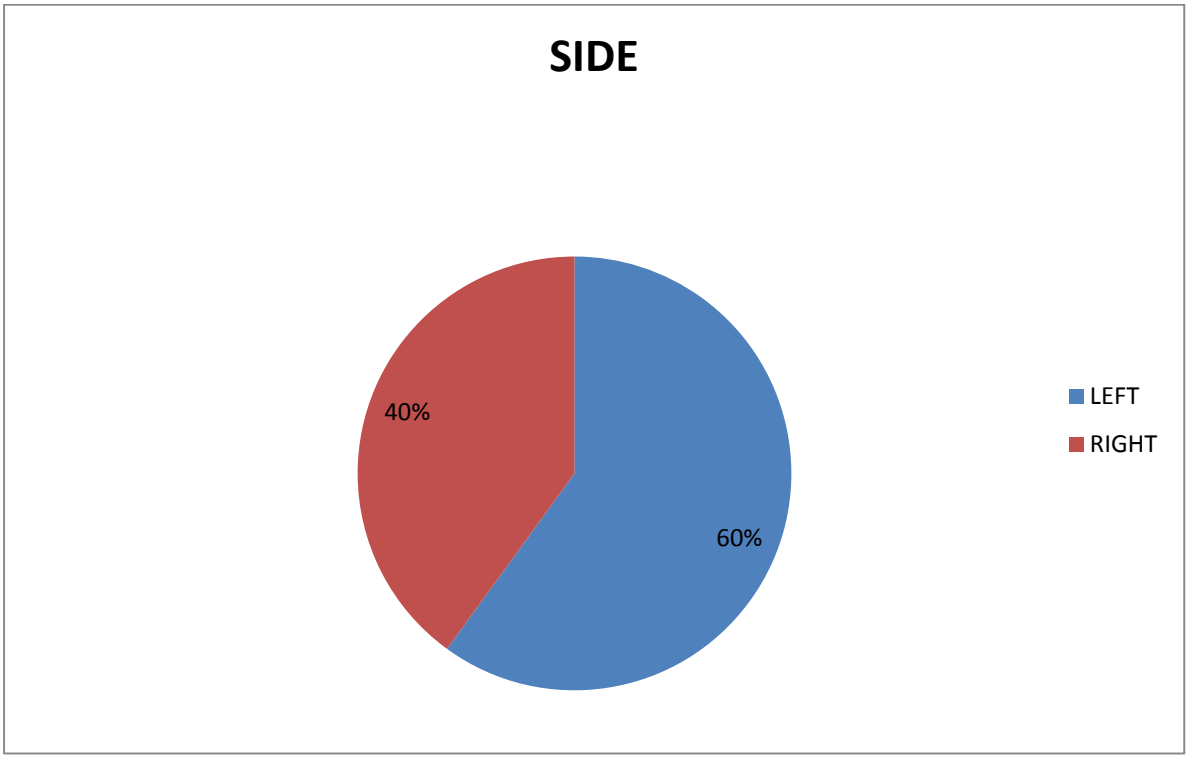


Table 4 : Distribution Of Size In Carcinoma Breast

Size Of The Tumor	Frequency	Percent
1-2CM	31	31.0
2-5CM	47	47.0
>5 CM	22	22.0
Total	100	100.0

This study showed that the size of the tumor were ranged from 1 cm to 10cm. Among 100 cases most of the tumor size ranged from 2-5 cm in size. 31 cases (31%) showed 1-2 cm size, 47 cases (47%) showed 2-5 cm size and 22 cases (22%) showed >5 cm size respectively.

Figure 6 : Distribution Of Size In Carcinoma Breast

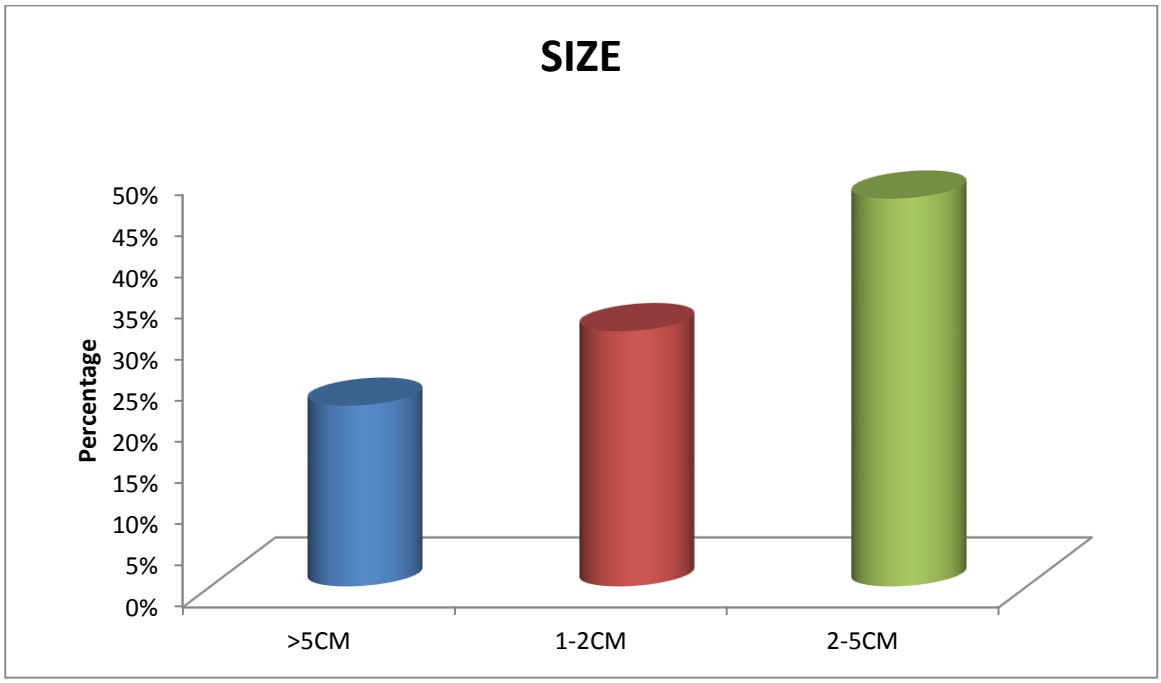


Table 5 : Distribution Of Histological Subtypes In Breast Cancer

DIAGNOSIS	N	%
IDC-NST	93	93.0%
IDC -medullary variant	1	1.0%
IDC with focal apocrine change	1	1.0%
IDC with focal medullary diff	1	1.0%
IDC with papillary features	1	1.0%
Medullary ca	1	1.0%
Metaplastic ca	1	1.0%
mucinous	1	1.0%
Total	100	100.0%

The commonest histological subtype in this study is IDC –NST type which constituted 93% of cases and other subtypes were medullary carcinoma 1 case, metaplastic carcinoma 1 case and mucinous carcinoma 1 case.

Table 6: Lymph Node Status in Carcinoma Breast

No. of nodes involved	Frequency	Percent
0-3	59	77.0
>3-9	14	18.0
>10	4	5.0
Total	67	100.0

In our study among 67 cases , 59 cases (77 %) had upto 3 nodes with metastatic carcinomatous deposits, 14 cases(18%) had 4 to 9 involved nodes,4 cases(5 %) had more than 10 involved nodes respectively.

Figure 7: Lymph Node Status in Carcinoma Breast

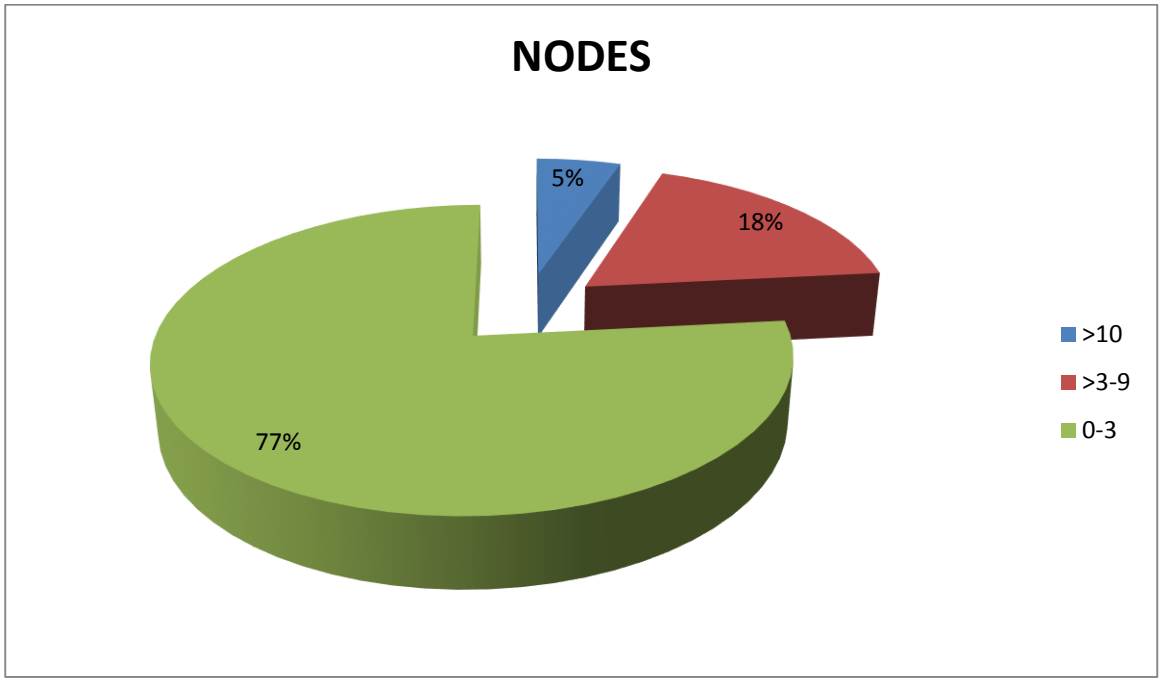


Table 7 : Histological Grade in Carcinoma Breast

Grade	Frequency	Percent
I	19	19.8
II	61	63.5
III	16	16.7
Total	96	100.0

Tumor grade was done according to modified Scarf Bloom Richardson grading system, 19 cases (19.8%) were Grade I, 61 cases (63.5%) were grade II and 16 Cases (16.7%) were grade III.

Figure 8 : Histological Grade in Carcinoma Breast

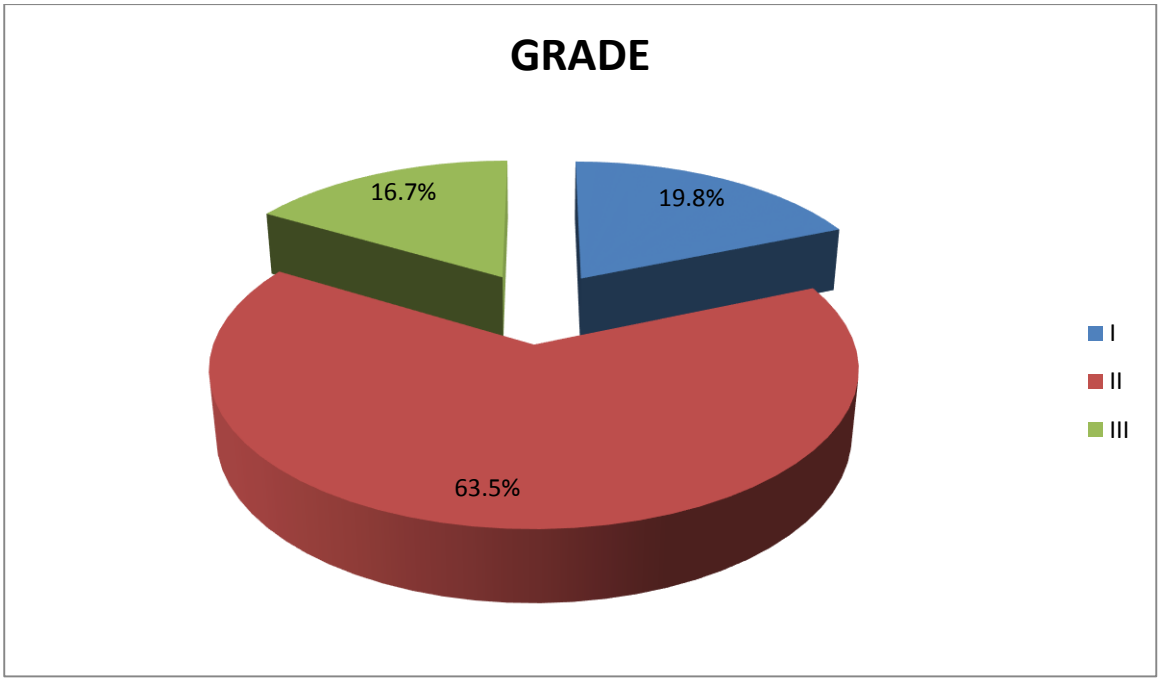
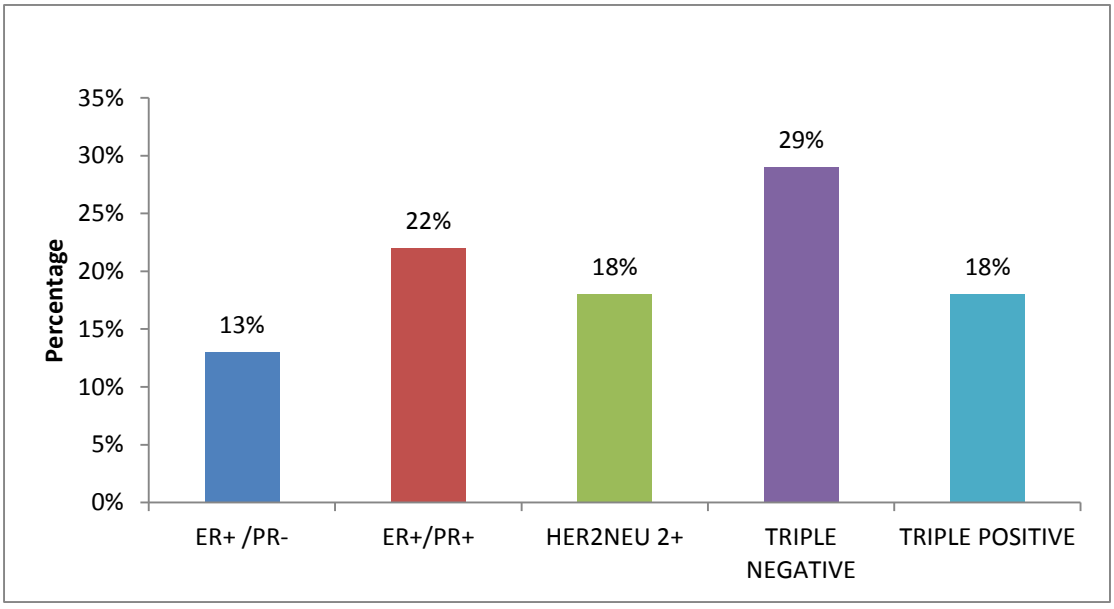


Table 8: Distribution of ER/ PR / Her2 Neu receptors in primary breast cancer

	Frequency	Percentage
ER +/ PR -	13	13%
ER + / PR+	22	22%
Her2 neu +	18	18%
Triple negative	29	29%
Triple positive	18	18%
Total	100	100%

In this study, IHC for ER , PR and HER2neu was done for 100 cases. Of which, 13 cases (13%) showed ER+ /PR-, 22 cases (22%) showed ER+ /PR+ , 18 Cases (18%) showed HER2 neu positive , 29 Cases (29%) showed triple negative and 18 cases (18%) showed triple positive.

Figure 9: Distribution of ER/ PR / Her2 Neu receptors in primary breast cncer



100 cases were evaluated for Tumor infiltrating lymphocytes by CD45 and CD 3 expression using Rabbit monoclonal and mouse polyconal Antibody (Pathnsitu- Ready to use) respectively. TILs are classified as three variables according to as per The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Lymph node is taken as control.

Table 9: Distribution of TILs in Breast Cancer

TILs %	No. of patients	%
0-10	16	16.0%
20-40	53	53.0%
50-90	31	31.0%
Total	100	100.0%

In this study, 16 % cases showed 0-10 % of stromal TILs , 53 % cases showed 20-40 % stromal TILs and 31 % cases Showed 50-90 % Stromal TILs

Table 10: Correlation of Tumor Infiltrating Lymphocytes and Age of the Patient

			TILS			Total
			0-10	20-40	50-90	
@__AGE	21-30	Count	0	0	1	1
		%within @___AGE	0.0%	0.0%	3.2%	1.0%
	31-40	Count	4	11	7	22
		%within @___AGE	25.0%	20.8%	22.6%	22.0%
	41-50	Count	4	17	13	34
		%within @___AGE	25.0%	32.1%	41.9%	34.0%
	51-60	Count	6	17	7	30
		%within @___AGE	37.5%	32.1%	22.6%	30.0%
	61-70	Count	1	8	3	12
		%within @___AGE	6.2%	15.1%	9.7%	12.0%
	71-80	Count	1	0	0	1
		%within @___AGE	6.2%	0.0%	0.0%	1.0%
Total		Count	16	53	31	100
		%within @___AGE	100.0%	100.0%	100.0%	100.0%

Pearson Chi-Square=10.544 P=0.394

In this study, Maximum percentage of 50-90 % stromal TILs are seen in the age group between 41-50 years. Age between 21-30 years and 71-80 years showed stromal TILs of 50-90% and 0-10% respectively. The non significant P value infers that TILs is independent of age of the patient.

Table 11: correlation of side of the tumor with TILs

		TILS			Total
		0-10	20-40	50-90	
SIDE Left	Count	11	30	19	60
	% within SIDE	68.8%	56.6%	61.3%	60.0%
right	Count	5	23	12	40
	% within SIDE	31.2%	43.4%	38.7%	40.0%
Total	Count	16	53	31	100
	% within SIDE	100.0%	100.0%	100.0%	100.0%

Pearson Chi-Square=0.787 P=0.675

Stromal TILs ranging from 50-90 % is Seen more on the left sided tumor than on the right side. Tumor Infiltrating Lymphocytes are not statistically significant with the side of the tumor.

Table 12: correlation of size of the tumor with TILs

			TILS			Total
			0-10	20-40	50-90	
SIZE	>5CM	Count	1	14	7	22
		% within TILS_NEW	6.2%	26.4%	22.6%	22.0%
	1-2CM	Count	9	13	9	31
		% within TILS_NEW	56.2%	24.5%	29.0%	31.0%
	2-5CM	Count	6	26	15	47
		% within TILS_NEW	37.5%	49.1%	48.4%	47.0%
Total		Count	16	53	31	100
		% within TILS_NEW	100.0%	100.0%	100.0%	100.0%

Pearson Chi-Square=6.691 P=0.153

In our study, size of tumor between 2-5 cm showed maximum stromal TILs of both 20- 40% and 50-90%.Tumor size between 1-2cm showed maximum of 0- 10% stromal TILs. The P- value was not significant.

Table 13: correlation of chemotherapy given with TILs

			TILS			Total
			0-10	20-40	50-90	
chemotherapy	Not given	Count	4	24	20	48
		%	8.3%	50.0%	41.7%	48.0%
	Given	Count	12	29	11	52
		%	31.2%	43.4%	38.7%	52.0%
Total		Count	16	53	31	100
		%	100.0%	100.0%	100.0%	100.0%

Pearson Chi-Square=6.94 P=0.05

In our study , P- value is statistically significant if neo-adjuvant chemotherapy given to the patient before Modified Radical Mastectomy.

			TILS			Total
			0-10	20-40	50-90	
DIAGNOSIS	IDC -medullary variant	Count	0	1	0	1
		% within DIAGNOSIS	0.0%	1.9%	0.0%	1.0%
	IDC with focal apocrine change	Count	0	1	0	1
		% within DIAGNOSIS	0.0%	1.9%	0.0%	1.0%
	IDC with focal medullary diff	Count	0	0	1	1
		% within DIAGNOSIS	0.0%	0.0%	3.2%	1.0%
	IDC with papillary features	Count	0	0	1	1
		% within DIAGNOSIS	0.0%	0.0%	3.2%	1.0%
	IDC-NST	Count	14	51	28	93
		% within DIAGNOSIS	87.5%	96.2%	90.3%	93.0%
	Medullary ca	Count	0	0	1	1
		% within DIAGNOSIS	0.0%	0.0%	3.2%	1.0%
	Metaplastic ca	Count	1	0	0	1
		% within DIAGNOSIS	6.2%	0.0%	0.0%	1.0%
	Mucinous	Count	1	0	0	1
		% within DIAGNOSIS	6.2%	0.0%	0.0%	1.0%
	Total	Count	16	53	31	100
		% within DIAGNOSIS	100.0%	100.0%	100.0%	100.0%

Pearson Chi-Square=19.086 P=0.162

Table 14: Correlation of histological subtypes with TILs

Most of the TILs for IDC-NST(51 cases) is ranging from 20-40%, 28 cases showed 50-90% TILs and 14 Cases showed 0-10% TILs. Medullary carcinoma showed 50- 90 % TILs, while metaplastic carcinoma and mucinous carcinoma showed only 0-10% TILs. The p- value is not statistically significant with the histological subtype.

Table 15: correlation of grading of tumor with TILS

			TILS			Total
			0-10	20-40	50-90	
GRADE	1	Count	2	9	8	19
		% within GRADE	14.3%	17.3%	26.7%	19.8%
	2	Count	12	34	15	61
		% within GRADE	85.7%	65.4%	50.0%	63.5%
	3	Count	0	9	7	16
		% within GRADE	0.0%	17.3%	23.3%	16.7%
Total		Count	14	52	30	96
		% within GRADE	100.0%	100.0%	100.0%	100.0%

Pearson Chi-Square=6.216 P=0.184

In this study, all the ranges of TILs (0-10%, 20-40%, 50-90%) are maximally seen in Grade 2 tumors. P- value is not statistically significant with the grading of tumor.

Table 16: correlating grading with TILs for neoadjuvant cases

			TILs			Total
			0-10	20-40	50-90	
GRADE	1	Count	2	2	1	5
		% within GRADE	20.0%	7.1%	8.3%	10.0%
	2	Count	8	21	7	36
		% within GRADE	80.0%	75.0%	58.3%	72.0%
	3	Count	0	5	4	9
		% within GRADE	0.0%	17.9%	33.3%	18.0%
Total		Count	10	28	12	50
		% within GRADE	100.0%	100.0%	100.0%	100.0%

Pearson Chi-Square=3.828* P=0.05

When TILs are compared with grading for those cases in which neo-adjuvant chemotherapy given , then P- value is statistically significant

Table 17: correlation of TILs with Lymph node status

			TILS		
			0-10	20-40	50-90
NODES	0-3	Count	11	28	20
		% within NODES	91.7%	70.0%	80.0%
	3-9	Count	1	8	5
		% within NODES	8.3%	20.0%	20.0%
	>10	Count	0	4	0
		% within NODES	0.0%	10.0%	0.0%
Total		Count	12	40	25
		% within NODES	100.0%	100.0%	100.0%

Pearson Chi-Square=5.079 P=0.279

In our study, when there is reduced number of nodes stromal TILs are increased, but lymph node status is not statistically significant with TILs

Table 18: correlation of TILs with ER+PR-/ER+PR+ breast tumor

		TILs			Total
		0-10	20-40	50-90	
ER+ /PR-	Count	2	6	5	13
	% within tils	33.3%	30.0%	55.6%	37.1%
ER+/PR+	Count	4	14	4	22
	% within tils	66.7%	70.0%	44.4%	62.9%
Total	Count	6	20	9	35
	% within tils	100.0%	100.0%	100.0%	100.0%

Pearson Chi-Square=1.781 P=0.410

In this study, ER+/ PR+ tumors showed more number of 0-10% and 20-40% Stromal TILs, while ER+/ PR- Tumors showed more number of 50-90% TILs.

Figure 10: correlation of TILs with ER+PR-/ER+PR+ breast tumor

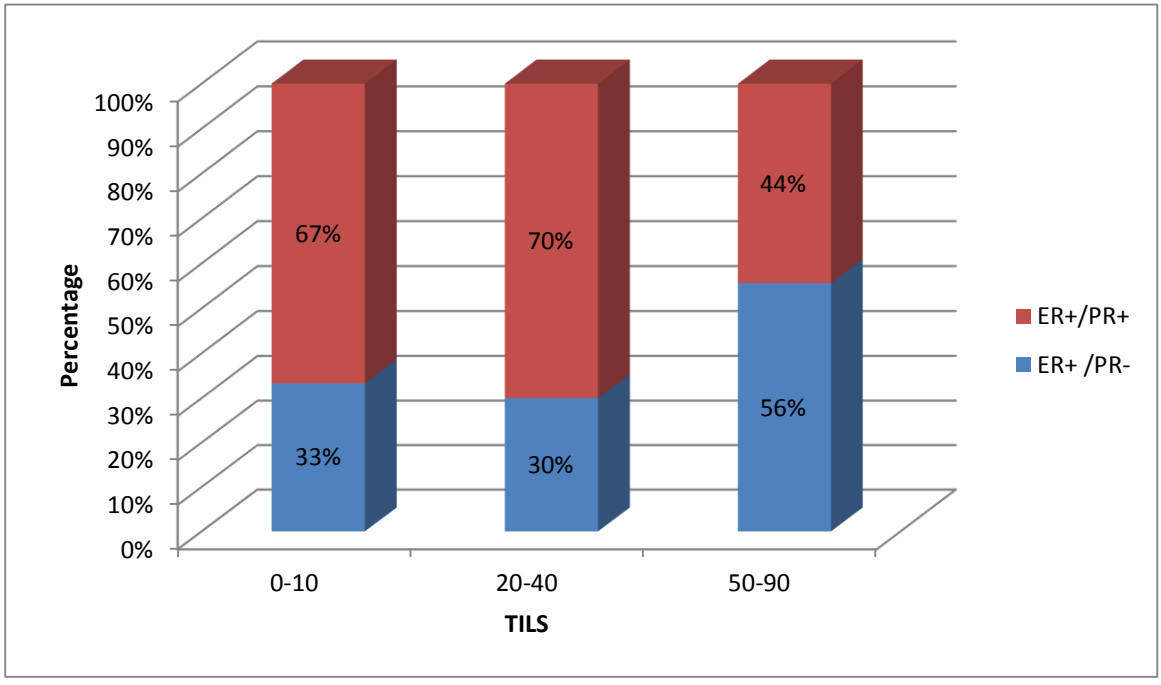


Table 19: correlation of TILs with ER+PR-/Her2neu breast tumors

		TILs			Total
		0-10	20-40	50-90	
ER+ /PR-	Count	2	6	5	13
	% within tils	33.3%	37.5%	55.6%	41.9%
HER 2NEU +	Count	4	10	4	18
	% within tils	66.7%	62.5%	44.4%	58.1%
Total	Count	6	16	9	31
	% within tils	100.0%	100.0%	100.0%	100.0%

Pearson Chi-Square=0.997 P=0.607

In this study, HER2neu positive tumors showed more number of 0-10% and 20-40% Stromal TILs, while ER+/ PR- Tumors showed more number of 50-90% TILS.

FIGURE 11: correlation of TILs with ER+PR-/Her2neu breast tumors

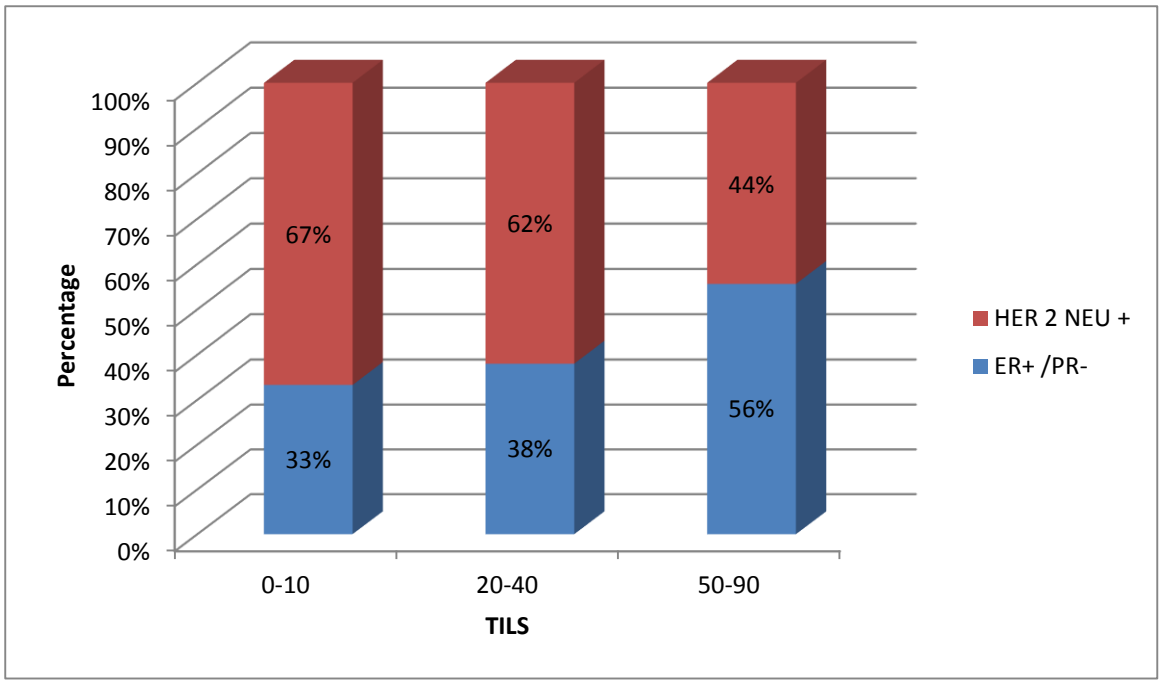


Table 20: correlation of TILs with ER+PR-/triple negative breast tumors

			TILs			Total
			0-10	20-40	50-90	
VAR00007	ER+ /PR-	Count	2	6	5	13
		% within tils	33.3%	28.6%	33.3%	31.0%
	TRIPLE NEGATIVE	Count	4	15	10	29
		% within tils	66.7%	71.4%	66.7%	69.0%
	Total	Count	6	21	15	42
		% within tils	100.0%	100.0%	100.0%	100.0%

Pearson Chi-Square=0.111 P=0.946

In this study, triple negative tumors showed more number of 0-10% , 20-40% and 50-90% TILs than ER+/PR- tumors

Figure 12: correlation of TILs with ER+PR-/triple negative breast tumors

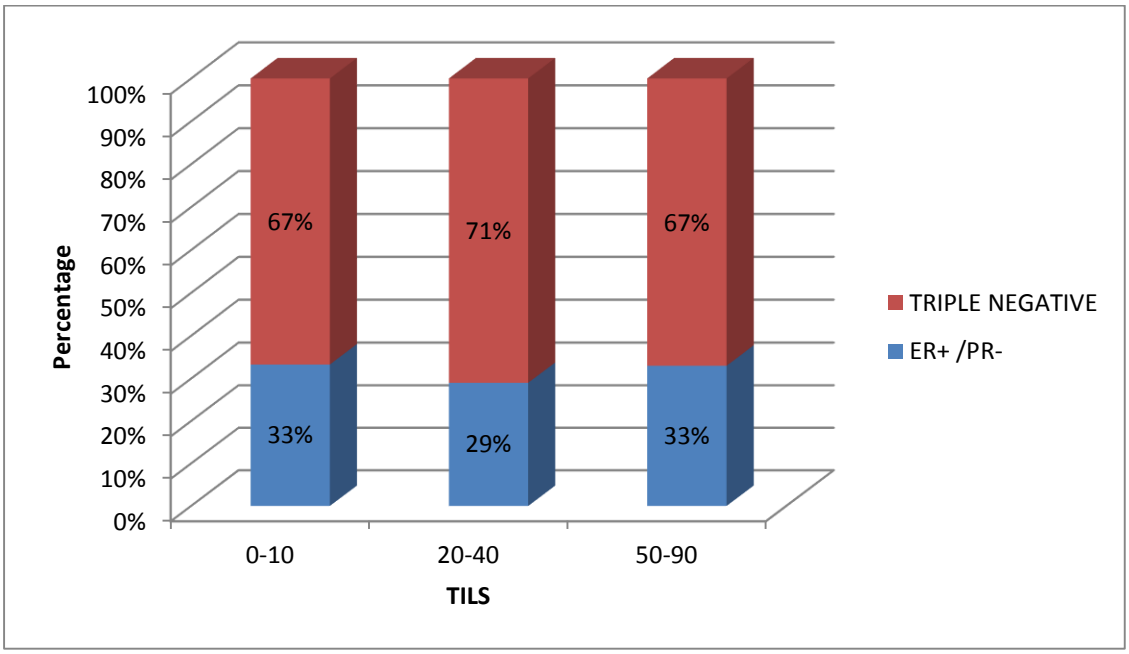


Table 21: correlation of TILs with ER+PR-/ triple positive breast tumors

			TILs			Total
			0-10	20-40	50-90	
VAR00007	ER+ /PR-	Count	2	6	5	13
		% within tils	50.0%	42.9%	38.5%	41.9%
	TRIPLE POSITIVE	Count	2	8	8	18
		% within tils	50.0%	57.1%	61.5%	58.1%
	Total	Count	4	14	13	31
		% within tils	100.0%	100.0%	100.0%	100.0%

Pearson Chi-Square=0.176 P=0.916

In this study, triple positive tumors showed more number of 0-10% ,20-40% and 50-90% Stromal TILs than ER+/ PR- Tumors .

Figure 13: correlation of TILs with ER+PR-/ triple positive breast tumors

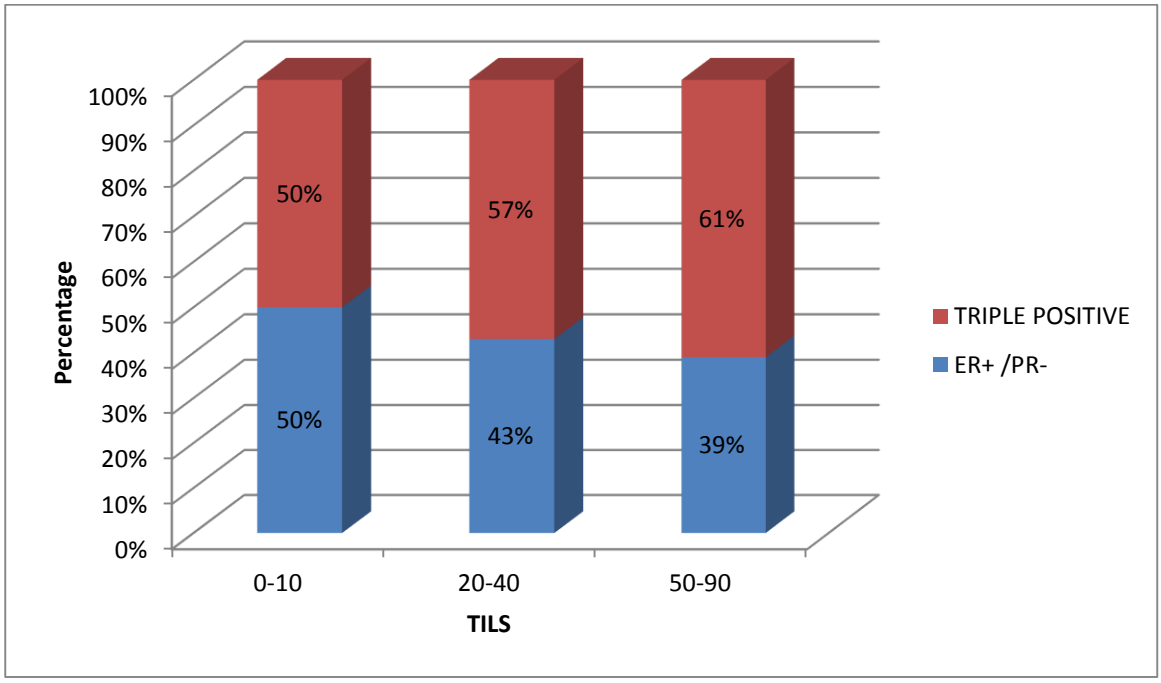


Table 22: correlation of TILs with ER+PR+/ triple negative breast tumors

			TILs			Total
			0-10	20-40	50-90	
VAR00007	ER+/PR+	Count	4	14	4	22
		% within tils	50.0%	48.3%	28.6%	43.1%
	TRIPLE NEGATIVE	Count	4	15	10	29
		% within tils	50.0%	51.7%	71.4%	56.9%
	Total	Count	8	29	14	51
		% within tils	100.0%	100.0%	100.0%	100.0%

Pearson Chi-Square= 1.677 P=0.432

In this study, triple negative tumors showed more number of 20-40%and 50-90% Stromal TILs than ER+/ PR +Tumors .while TILs ranging between 0-10% is equally distributed between Triple negative and ER+/PR+ tumors.

Figure 14: correlation of TILs with ER+PR+/ triple negative breast tumors

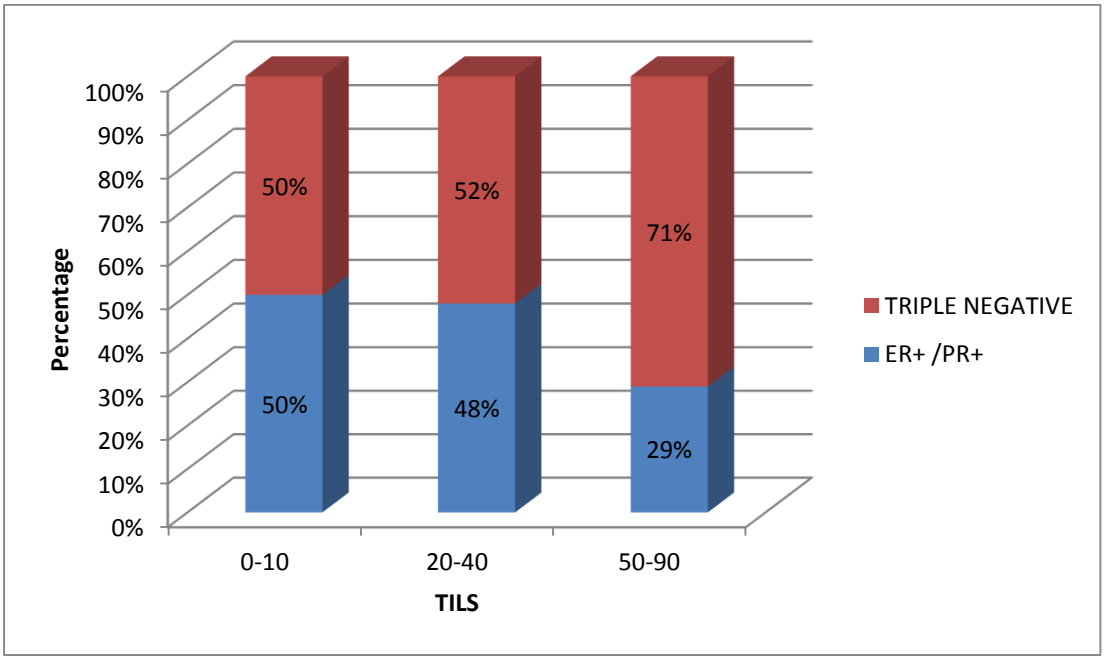


Table 23: correlation of TILs with ER+PR+ / triple positive breast tumors

.			TILs			Total
			0-10	20-40	50-90	
VAR00007	ER+/PR+	Count	4	14	4	22
		% within tils	66.7%	63.6%	33.3%	55.0%
	TRIPLE POSITIVE	Count	2	8	8	18
		% within tils	33.3%	36.4%	66.7%	45.0%
	Total	Count	6	22	12	40
		% within tils	100.0%	100.0%	100.0%	100.0%

Pearson Chi-Square= 3.269 P=0.195

In this study, ER+/ PR+ tumors showed more number of 0-10% and 20-40% Stromal TILs, while triple positive Tumors showed more number of 50-90% TILs.

Figure 15: correlation of TILs with ER+PR+ / triple positive breast tumors

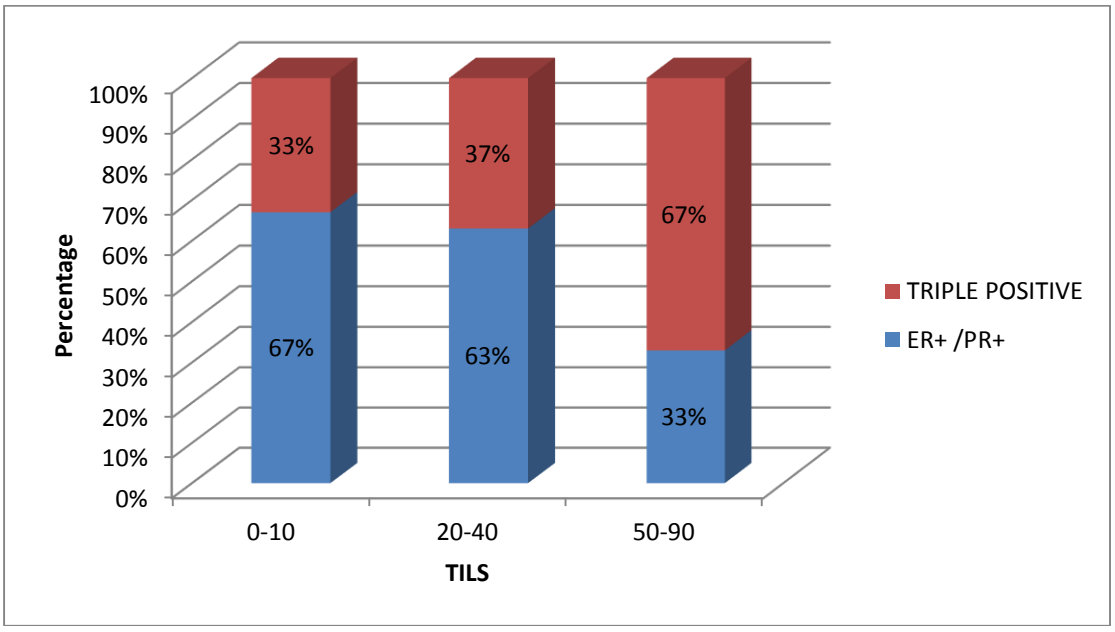


Table 24: correlation of TILs with ER+PR+/ HER2neu breast tumors

			TILs			Total
			0-10	20-40	50-90	
VAR00007	ER+/PR+	Count	4	14	4	22
		% within tils	50.0%	58.3%	50.0%	55.0%
	HER2NEU 2+	Count	4	10	4	18
		% within tils	50.0%	41.7%	50.0%	45.0%
	Total	Count	8	24	8	40
		% within tils	100.0%	100.0%	100.0%	100.0%

PearsonChi-Square=0.269P=0.874

ER+/ PR+ tumors and HER2neu positive tumors shows equally distributed TILs in 0-10% and50-90% group, with mild increase of tils seen between 20-40% in ER+/PR+ tumors.

Figure 16: correlation of TILs with ER+PR+/ HER2neu breast tumor

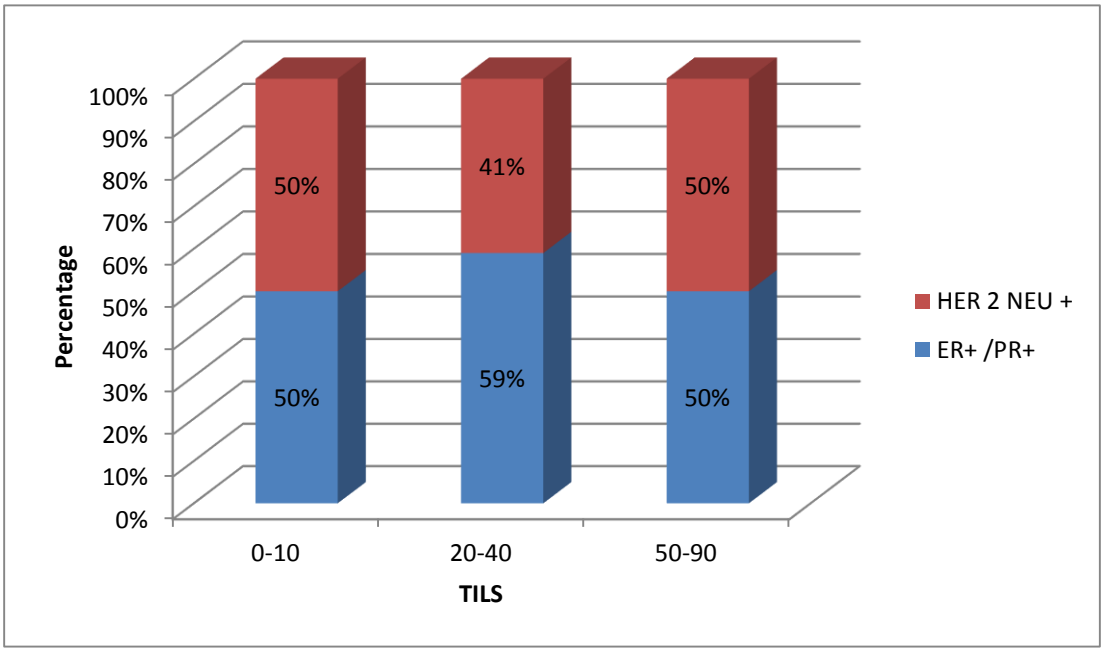


Table 25: correlation of TILs with HER2neu/ triple negative breast tumors

			tils			Total
			0-10	20-40	50-90	
VAR00007	HER2NEU 2+	Count	4	10	4	18
		% within tils	50.0%	40.0%	28.6%	38.3%
	TRIPLE NEGATIVE	Count	4	15	10	29
		% within tils	50.0%	60.0%	71.4%	61.7%
	Total	Count	8	25	14	47
		% within tils	100.0%	100.0%	100.0%	100.0%

Pearson Chi-Square= 1.055 P=0.590

In this study,HER2neu tumors showed more cases of 20-40% and 50-90% Stromal TILs than triple negative tumors.

Figure 17: correlation of TILs with HER2neu/ triple negative breast tumors

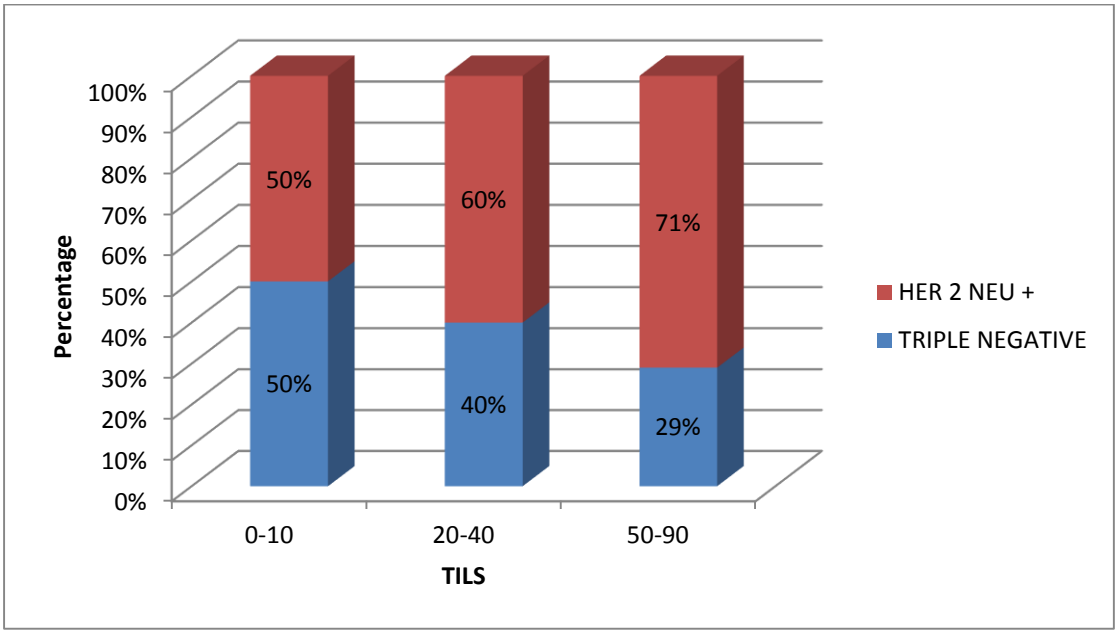


Table 26: correlation of TILs with HER2neu + / triple positive breast tumors

			Tils			Total
			0-10	20-40	50-90	
VAR00007	HER2NEU 2+	Count	4	10	4	18
		% within tils	66.7%	55.6%	33.3%	50.0%
	TRIPLE POSITIVE	Count	2	8	8	18
		% within tils	33.3%	44.4%	66.7%	50.0%
	Total	Count	6	18	12	36
		% within tils	100.0%	100.0%	100.0%	100.0%

Pearson Chi-Square= 2.222 P=0.329

In this study, her2neu tumors showed more cases of 0-10% and 20-40% Stromal TILs, while triple positive Tumors showed more cases of 50-90% TILs.

Figure 18: correlation of TILs with HER2neu + / triple positive breast tumors

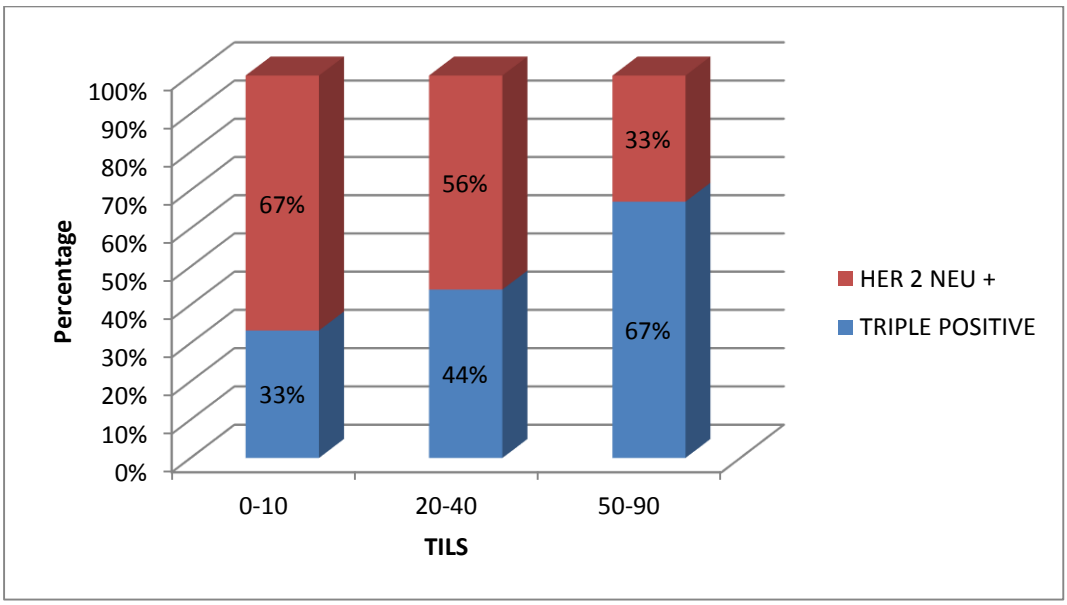


Table 27: correlation of TILs with triple negative/ triple positive breast tumors

			tils			Total
			0-10	20-40	50-90	
VAR00007	TRIPLE NEGATIVE	Count	4	15	10	29
		% within tils	66.7%	65.2%	55.6%	61.7%
	TRIPLE POSITIVE	Count	2	8	8	18
		% within tils	33.3%	34.8%	44.4%	38.3%
	Total	Count	6	23	18	47
		% within tils	100.0%	100.0%	100.0%	100.0%

Pearson Chi-Square= 0.477 P=0.790

In this study, triple negative tumors showed more cases of 0-10%, 20-40% and 50-90%Stromal TILs than triple positive tumors.

Table 19: correlation of TILs with triple negative/ triple positive breast tumors

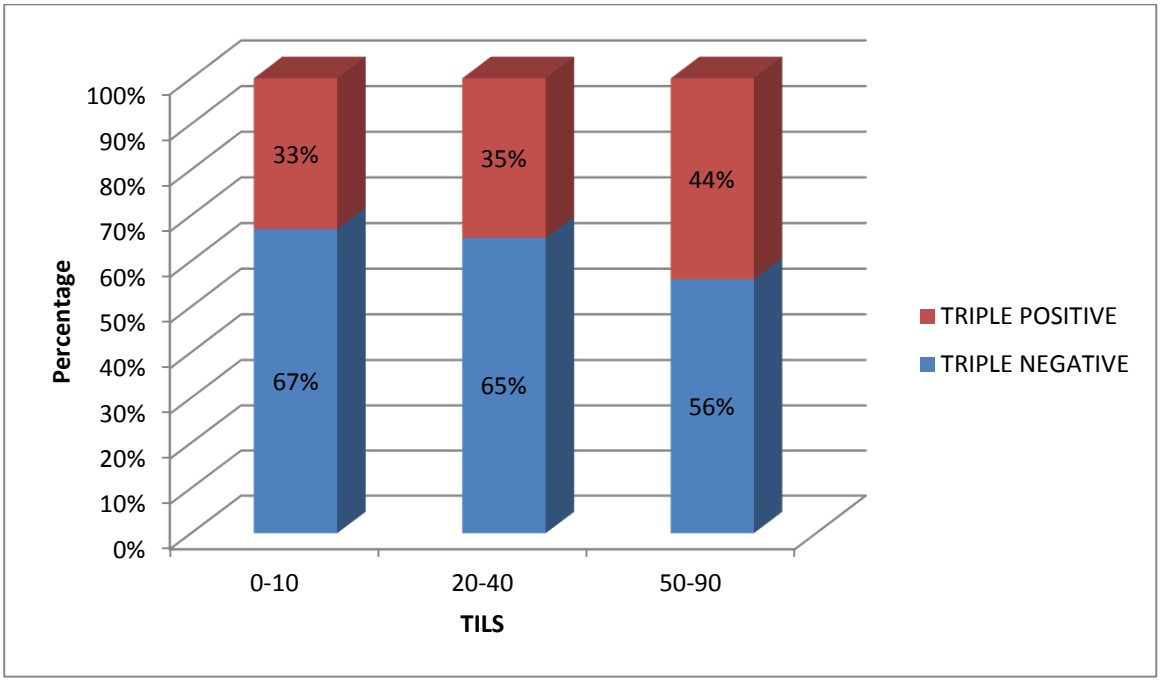


Table 28: correlation of TILs with ER/PR/HER2neu breast tumors

			TILs			Total
			0-10	20-40	50-90	
	ER+ /PR-	Count	2	6	5	13
		% within tils	12.5%	11.3%	16.1%	13.0%
	ER+/PR+	Count	4	14	4	22
		% within tils	25.0%	26.4%	12.9%	22.0%
	HER2NEU 2+	Count	4	10	4	18
		% within tils	25.0%	18.9%	12.9%	18.0%
	TRIPLE NEGATIVE	Count	4	15	10	29
		% within tils	25.0%	28.3%	32.3%	29.0%
	TRIPLE POSITIVE	Count	2	8	8	18
		% within tils	12.5%	15.1%	25.8%	18.0%
Total		Count	16	53	31	100
		% within tils	100.0%	100.0%	100.0%	100.0%

Pearson Chi- Square= 4.735, P= 0.785

Even though there is strong association with triple negative and ER+/PR+ tumors with tumor infiltrating lymphocytes in this study, P- value is not Statistically significant.

DISCUSSION

DISCUSSION

Breast carcinoma is the most common cancer in the urban Indian women and it is the second most common cancer in the rural women. The annual incidence varies from 5/100,000 population in rural areas to 30/100,000 population in urban areas.

Carcinoma breast is a heterogenous disease both clinically and pathologically. Mortality of breast cancer can be reduced by early detection, appropriate management and targeted therapies. Apart from the prognostic markers like stage,grade, lymphnode status, ER,PR, HER2, there are many new theories studied for prognosis. One such attempt was tumor infiltrating lymphocytes.

Therefore evaluation of TILs might provide information regarding the biological profile and may help in evaluation of patients immune response to the tumor.

In the present study, tumor infiltrating lymphocytes in the breast cancer was assessed by counting stromal TILs microscopically and IHC evaluation done by using CD45 and CD3 and an attempt was made to correlate the TILs with clinicopathological factors.

This study showed that the highest incidence of breast carcinoma occurred in 41- 50years age group. This correlates with the Adenji et al with peak age group of 41-50 years.(73). The age of breast carcinoma ranged from

26 years to 75 years with the median age of presentation is 50 years. This correlates with raina et al, median age of incidence is 47 years.(74).

In our study, 1% are in 21-30 years of age, 22% are in 31- 40 years of age and 31 % are in 41-50 years of age. According to National Cancer Registry Programme in 2012 – 2014 , 4 % were in 21-30 years of age group, 16 % were in 31 -40 years of age and 28% were in 41-50 years of age. 48 % patients were in the below 50 years of age , while in our study, 57 % patients are in below 50 years of age. So, there is a increasing trend of breast cancer in young population.

Table 29: Comparision Of Age Of Patient In Bresat Cancer

Age group	Current study	National cancer registry programme
21-30	1	4
31-40	22	16
41-50	34	28
51-60	30	30
61-70	13	22
Total	100	100.0

The most common side of breast carcinoma in this study was left side of breast in 60% cases, while the right side is about 40% cases. In our study, 20 % more cases are common on left side breast than right breast. This correlated with Tulinius H et al shows excess 13 % cases on left side breast.(24).

The size of the tumor ranged from 1-10cm. among the 100 cases, most of the tumor size ranged between 2-5cm size (47%) cases, 31% cases were 1-2 cm in size and 22% showed > 5cm in size. According to Freitas Junior et al 9.1 % cases were in 1-2 cm tumor size, 33 % cases were in 2-5cm tumor size and 25.8 % cases were in > 5 cm tumor size.(75).

Table 30: Comparision Of Size Of The Tumors

Size of the tumor	Freitas junior et al	Current study
1-2 CM	9.1%	31%
2-5 CM	33%	47%
>5CM	25.8%	22%

Among the histological subtypes, invasive ductal carcinoma- NST type comprised the most common with 93%, mucinous carcinoma 1% medullary carcinoma 1% and metaplastic carcinoma 1%. Albreksten et al showed that IDC- NST- 81.4 %, Mucinous Carcinoma- 1.5% and medullary carcinoma- 1.1%.(76).

Table 31: Comparision Of Histological Subtyping Of The Tumors

Histological subtypes	Albreksten et al	Current study
IDC- NST	81.4 %	93%
MEDULLARY CARCINOMA	1.1%	1%
MUCINOUS CARCINOMA	1.5%	1%
METAPLASTIC CARCINOMA	-	1%

The grade II tumors were more common than the other grades. Tumor grade was done according to modified Scarf Bloom Richardson grading system, 19 cases (19.8%) were Grade I, 61 cases (63.5%) were grade II and 16 Cases (16.7%) were grade III. Thomas et al (2009) (77) and Blamey et al (2009) (78) showed 45 % and 41 % of grade II tumors respectively.

Table 32: Comparision Of Grading Of Tumors

Grade	Thomas et al	Blamey et al	Current study
GRADE I	26%	29%	19.8%
GRADE II	45%	41%	63.5%
GRADE III	29%	30%	16.7%

Among the axillary lymph node dissection along with the mastectomy specimen , 77 % cases showed upto 3 nodes with metastatic carcinomatous deposits, 18% showed 4 to 9 involved nodes and 5 % showed more than 10 involved nodes .

Molecular study done in this study showed 13% of ER+ /PR- cases, 22% of ER+ /PR+ cases, 18% of HER2 neu positive cases , 29% of triple negative cases and 18% of triple positive cases.

As per Salgado et al, this study shows 0-10 % of stromal TILs in 16% cases , 20-40 % stromal TILs in 53 % cases and 50-90 % Stromal TILs in 31% cases.(72)

The median age of our study was 50 years of age. Maximum percentage of 50-90 % stromal TILs are seen in the age group between 41-50 years. Age between 21-30 years and 71-80 years showed stromal TILs of 50-90% and 0-10% respectively. There was no significant correlation found between age of the patient and TILs (P-value: 0.394). Sasha E. Stanson et al shows high lymphocytic infiltrate was associated with young age.(54)

In our study, 50- 90 % stromal TILs are seen more on the left sided tumor than on the right side. The side of the breast involved had no correlation with Tumor Infiltrating Lymphocytes (P value- 0.675).

The stromal TILs of 20-40% and 50- 90% are maximally seen in the tumor size of 2-5cm and maximum of 0-10% are seen in 1-2 cm tumor size. In our study, tumor size did not correlate with TILs (P value – 0.153). According to H.Othani et al, T1 and T2 tumors (1-2cm, 2-5 cm size respectively) showed maximum Tumor Infiltrating Lymphocytes.(79).

In our study, neo-adjuvant chemotherapy given before Modified Radical Mastectomy has correlated with Tumor Infiltrating Lymphocytes (P value - 0.05). Ke wang et al says chemotherapy-induced cell death, which releases tumor antigens that can be taken up and processed by antigen presenting cells (APCs) to T cells, leading to the direct and destroy cancer cells by activated T cells and there is increase in Lymphocytes following chemotherapy.(80)

In our study, 51% of cases showed TILs of 20-40%, 28 % cases showed 50- 90 % TILs and 14 % cases showed TILs of 0-10%. 1% case of medullary carcinoma shows 50-90% TILs, 1% case of metaplastic carcinoma shows 0-

10% TILs and 1% case of mucinous carcinoma shows 0-10% TILs. Histological subtying of breast cancers are not correlated with TILs (P value- 0.162).

In our study, Tumor Infiltrating Lymphocytes are maximally seen in grade II tumors. Grading of tumor does not correlate with Tumor Infiltrating Lymphocytes (P- Value : 0.184). H. Ohtani et al showed 0 % in grade I, 8.3 % in grade II tumors, 29 % in grade III tumors.(79)

When grading of tumors are compared for those cases in which prior chemotherapy given in this present study have correlated with Tumor Infiltrating lymphocytes. (P value- 0.05)

In this study, stromal TILs are increased if there is reduced number of axillary lymph nodes showing metastatic deposits. Lymph node status had no correlation with Tumor Infiltrating Lymphocytes. (P=0.279)

In our study, ER+/ PR+ tumors showed more number of 0-10% and 20-40% Stromal TILs, while ER+/ PR- Tumors showed more number of 50-90% TILs. Tumor infiltrating Lymphocytes had no correlation with ER/ PR status (P=0.410).

In this present study, HER2neu positive tumors showed more number of 0-10% and 20-40% Stromal TILs, while ER+/ PR- Tumors showed more number of 50-90% TILs. ER/PR/ HER2neu status had no correlation with TILS (P=0.607)

In this study, triple negative tumors showed more number of 0-10% , 20-40% and 50-90% TILs than ER+/PR- tumors. Tumor Infiltrating Lymphocytes had no correlation with the ER/PR/HER2neu status. (P=0.946).

In this study, triple positive tumors showed more number of 0-10% ,20-40% and 50-90% Stromal TILs than ER+/ PR- Tumors . ER/PR/HER2neu status had no correlation with Tumor Infiltrating Lymphocytes. (P=0.916).

In our study, triple negative tumors showed more number of 20-40%and 50-90% stromal TILs than ER+/ PR+ Tumors .while TILs ranging between 0-10% is equally distributed between Triple negative and ER+/PR+ tumors. Tumor infiltrating Lymphocytes does not correlate with ER/PR/HER2neu status (P=0.432).

In this present study, ER+/ PR+ tumors showed more number of 0-10% and 20-40% Stromal TILs, while triple positive Tumors showed more number of 50-90% TILs. There is no correlation between tumor infiltrating lymphocytes and ER/ PR/ HER2neu (P=0.195).

In this study, ER+/ PR+ tumors and HER2neu positive tumors shows equally distributed TILs in 0-10% and50-90% group, with mild increase of tils seen between 20-40% in ER+/PR+ tumors. The Stromal TILs are not significantly correlated with the ER/ PR/ HER2neu status. (P=0.874).

In this study,HER2neu tumors showed more cases of 20-40% and 50-90% Stromal TILs than triple negative tumors. No significant correlation between TILS and ER/PR/HER2neu status (P=0.590)

In this study, HER2neu positive tumors showed more cases of 0-10% and 20-40% Stromal TILs, while triple positive tumors showed more cases of 50-90% TILs. Stromal TILs had no correlation with ER/PR/HER2neu status (P=0.329).

In this study, triple negative tumors showed more cases of 0-10%, 20-40% and 50-90% stromal TILs than triple positive tumors. There is no correlation with Tumor Infiltrating Lymphocytes and ER/PR/HER2neu status (P=0.790)

In our study, most of cases are triple negative and next most common is ER+ /PR + positive tumors in which there was strong association between tumor infiltrating lymphocytes and Triple Negative tumors and ER/PR positive tumors. But ,there is no correlation between triple negative breast tumors and Tumor Infiltrating Lymphocytes (P=0.785).

Many studies showed that Triple negative tumors and HER2neu positive tumors have increase in stromal TILs both in and around the tumor. Sasha E. Stanton et al showed that there is incremental increase in and around the tumor in triple negative and HER2 neu positive tumors.(54). According to H. Othani et al , if there is decrease in tumor infiltrating lymphocytes in triple negative breast cancer and HER2neu positive tumors is considered as high grade.(79).

Sasha E. Stanton et al showed that triple negative tumors have correlated well with Tumor infiltrating Lymphocytes (p= 0.023). But in our study, Tumor infiltrating lymphocytes had no correlation with triple negative tumors.(72).

LIMITATIONS OF THIS STUDY:

- Through these discussions, limitations of the present study are also noted.
- In the present study, the cases were selected on the basis of histopathological classification in the tertiary care centre and not a population based study, which will not reflect the true prevalence of the general population.
- Due to economic constraints, only 100 cases are included in this present study.

SUMMARY

SUMMARY

This study is conducted in the Institute of Pathology, Madras Medical College, Chennai during the period between January 2013 to December 2015. It is a retrospective study. Out of the 2083 breast specimens, breast malignancies constitutes for 45.2% of all cases.

Detailed history regarding Patient's age, sex, side of the breast involved, Grade, Lymph node involvement, neo-adjuvant chemotherapy and Hormonal status like status of ER, PR, HER2neu were assessed for 100 cases. Tumor Infiltrating Lymphocytes are evaluated with IHC CD45 and CD3 for 100 cases, in which 93% cases were Infiltrating Ductal carcinoma-NOS cases and 07 % cases are of special types like Mucinous, Metaplastic, Apocrine, and Medullary carcinoma.

Tumor infiltrating lymphocytes were evaluated and IHC CD45 and CD3 was done in these cases. Slides were evaluated and scoring was done by Salgado et al scoring system and results were compared with other Histopathological parameters and Hormonal receptors like ER, PR and HER2neu status.

- Highest incidence of breast carcinoma occur in the age group of 41-50 years.
- Infiltrating Ductal Carcinoma NOS is the most common primary malignant neoplasm of breast constituting 93% of cases.
- Most of the malignant tumors are left sided.
- Forty seven percent of cancers are presented in the size range of 2-5cms.

- Majority of the tumors are of Grade II accounting for 63.5% of all cases.
- Notably 77 % cases presented with Lymph node metastasis and majority of them with N1 stage.
- Estrogen receptor expression was observed in 53 % cases and Progesterone receptor was observed in 40 % cases and 36 % of cases were positive for HER2neu.
- 13% cases showed ER+ /PR-, 22% cases showed ER+ /PR+ , 18% cases showed HER2 neu positive , 29% cases showed triple negative and 18% cases showed triple positive.
- 0- 10 % stromal TILs are in 16 % cases , 20 – 40 % stromal TILs are seen in 53% and 50 -90 % stromal TILs are seen in 31 % .
- 50 – 90 % of stromal TILs are seen maximum in the age group of 41- 50 years of about 41.9 % , though it was not statistically significant.
- The size and side of the tumor was not statistically significant.
- Fifty two cases of neo-adjuvant chemotherapy were included in this study, neo-adjuvant chemotherapy was statistically significant with Tumor Infiltrating Lymphocytes.
- Of the IDC-NST ,51 cases is ranging from 20-40%, 28 cases showed 50-90% TILs and 14 Cases showed 0-10% TILs. Medullary carcinoma showed 50- 90 % TILs, while metaplastic carcinoma and mucinous carcinoma showed only 0-10% TILs. Histological subtyping has no correlation with TILs.
- Grading of tumors does not correlate with TILs.

- But grading of tumors correlate with TILs when neo-adjuvant chemotherapy given cases are taken into consideration.
- Lymph node status does not correlate with TILs.
- Each of ER+/PR+ / ER+PR- /her2NEU +/ Triple positive / Triple Negative tumors are compared with the all the variables (0- 10 %, 20 – 40 %, 50 – 90 %) of Tumor infiltrating Lymphocytes.
- The present study showed that Triple negative tumors had strong association with Tumor Infiltrating Lymphocytes. This is in concurrence with many studies.

CONCLUSION

CONCLUSION

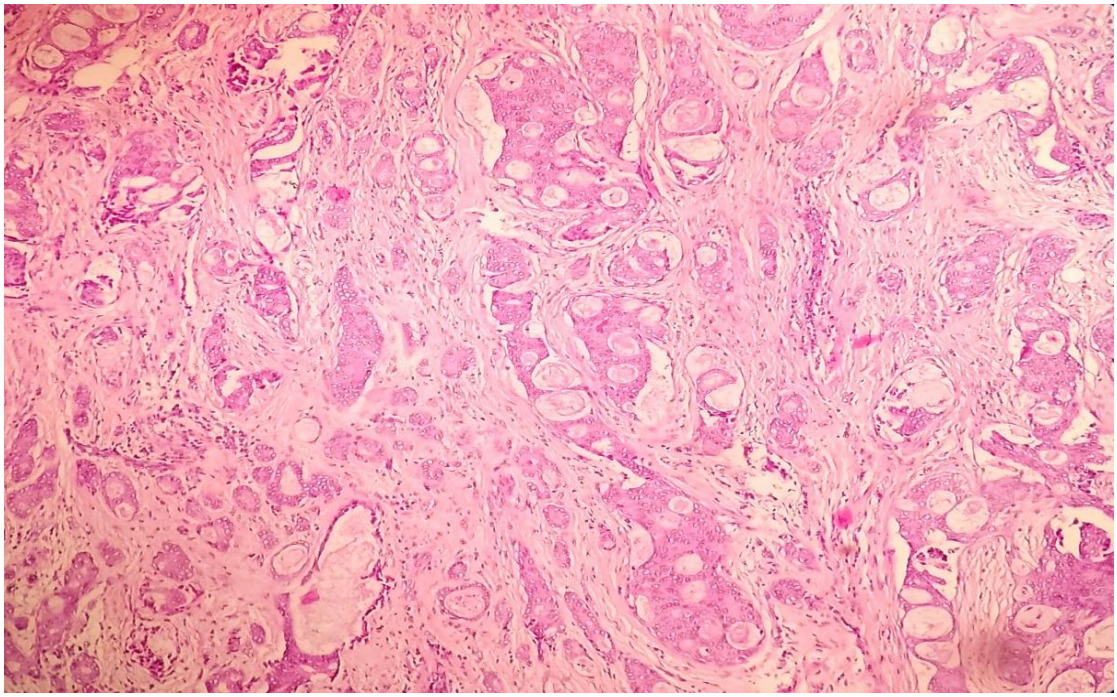
- In our study, among the breast specimens received, Malignant breast tumors constituted to 45.2 % .
- The stromal TILs are classified as 0- 10 %, 20 -40 % and 50- 90 % as per Salgado et al.
- Younger age group show increase number of TILs.
- Maximum of stromal TILs are of 50- 90 % are seen in IDC- NST type next to Medullary carcinoma.
- Less number of nodes show increase number of TILs.
- TILs are not related with side and size of the tumor.
- Fifty two cases of neo- adjuvant chemotherapy given cases and its grading had significant correlation with TILs.
- Among the ER,PR, HER2neu status , most of the TILs were expressed in triple negative tumors. However a larger study is required for the evaluation of the same.

COLOR PLATES

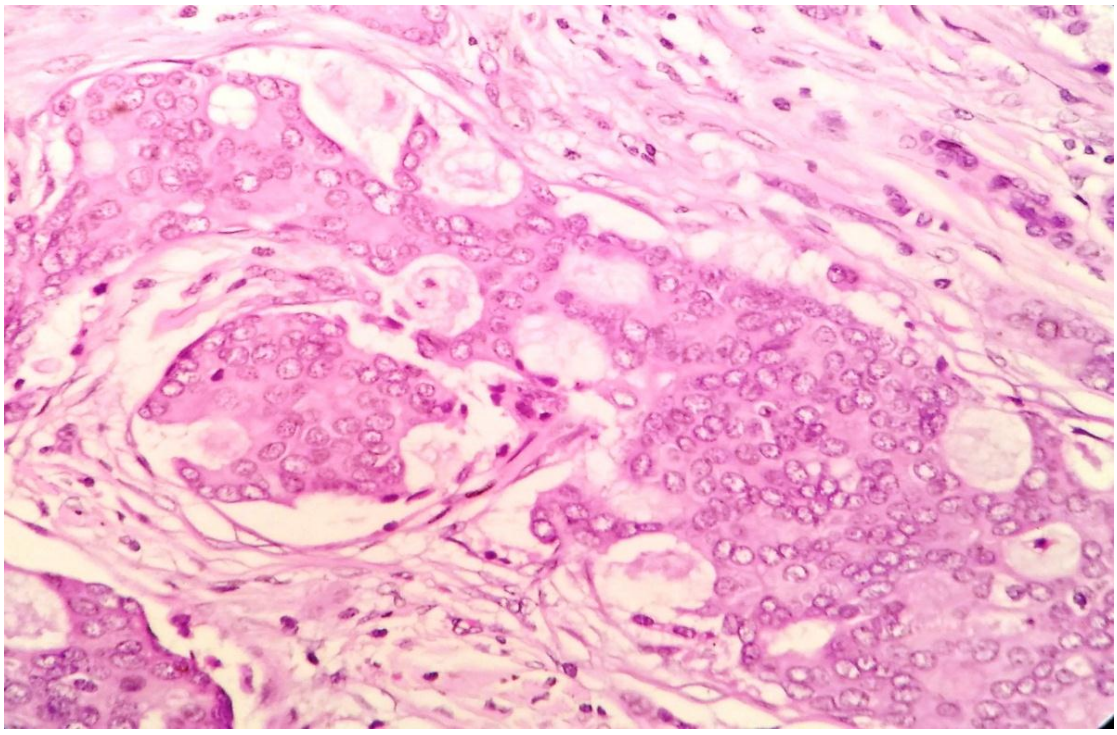
DUCTAL CARCINOMA BREAST

BX-3854/15 : Firm grey white growth with irregular margins

INVASIVE DUCTAL CARCINOMA NOS – GRADE 1

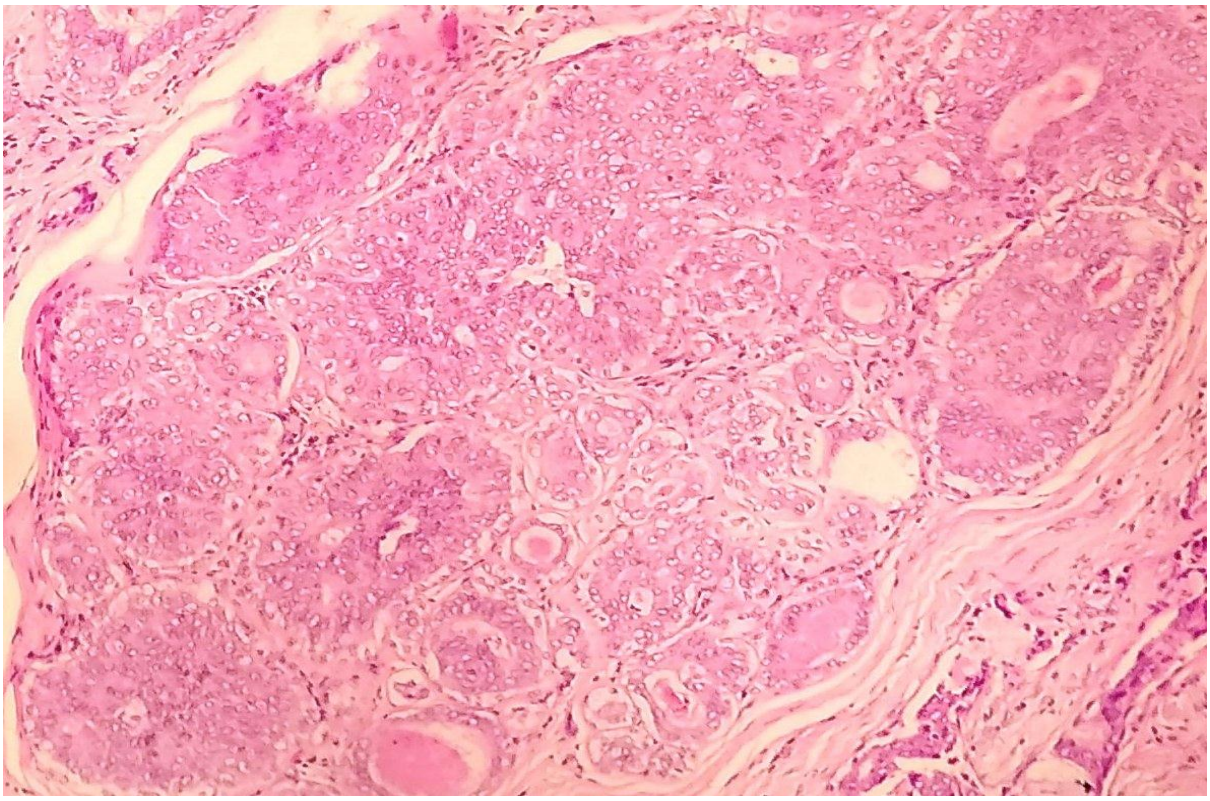


BX 2849/15- Invasive ductal carcinoma NOS >75 % tumor cells show tubule formation (100X , H &E)

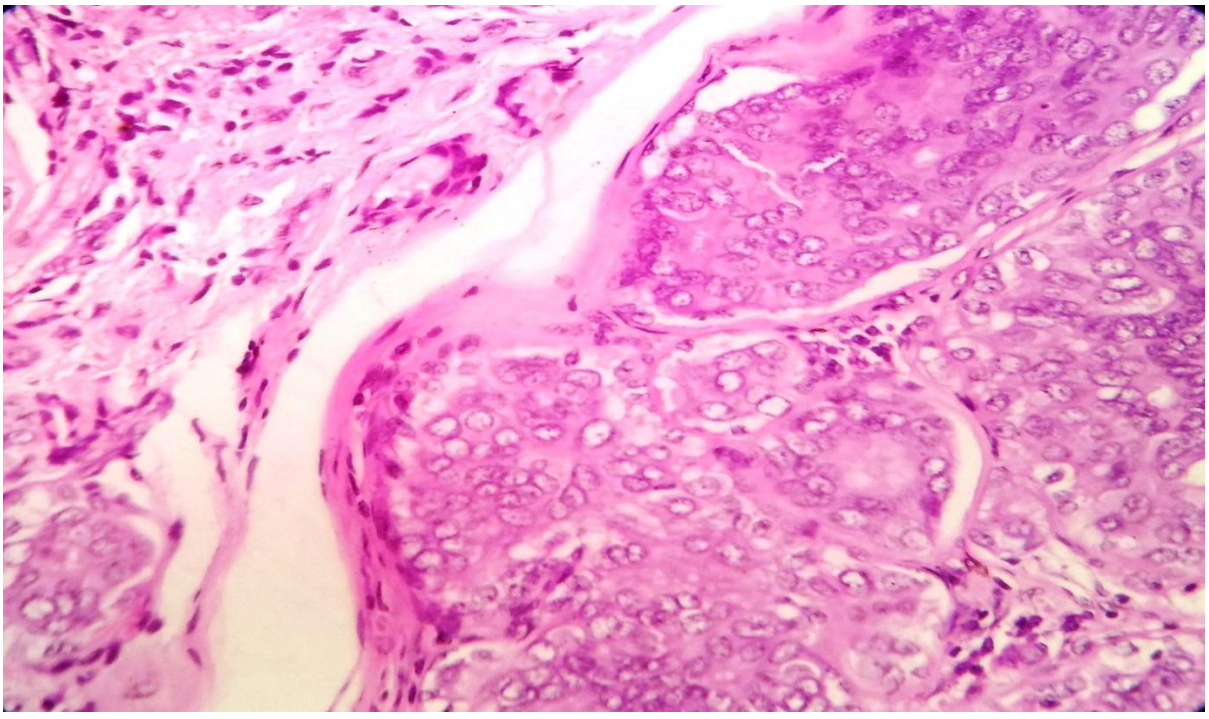


BX 2849/15- Malignant duct epithelial cells with mild nuclear pleomorphism (400X , H& E)

INVASIVE DUCTAL CARCINOMA NOS -GRADE II

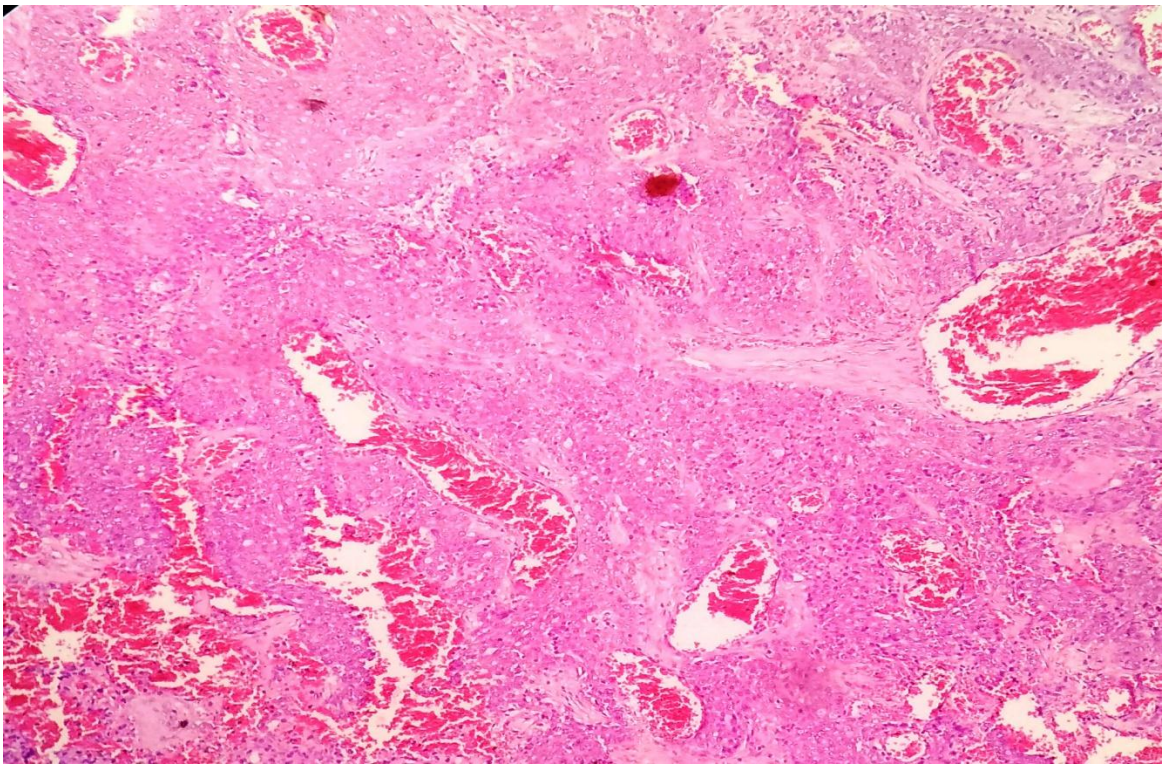


BX 5127 /15 –Sheets of malignant epithelial cells ,30 % tubule formation (100 X , H& E)

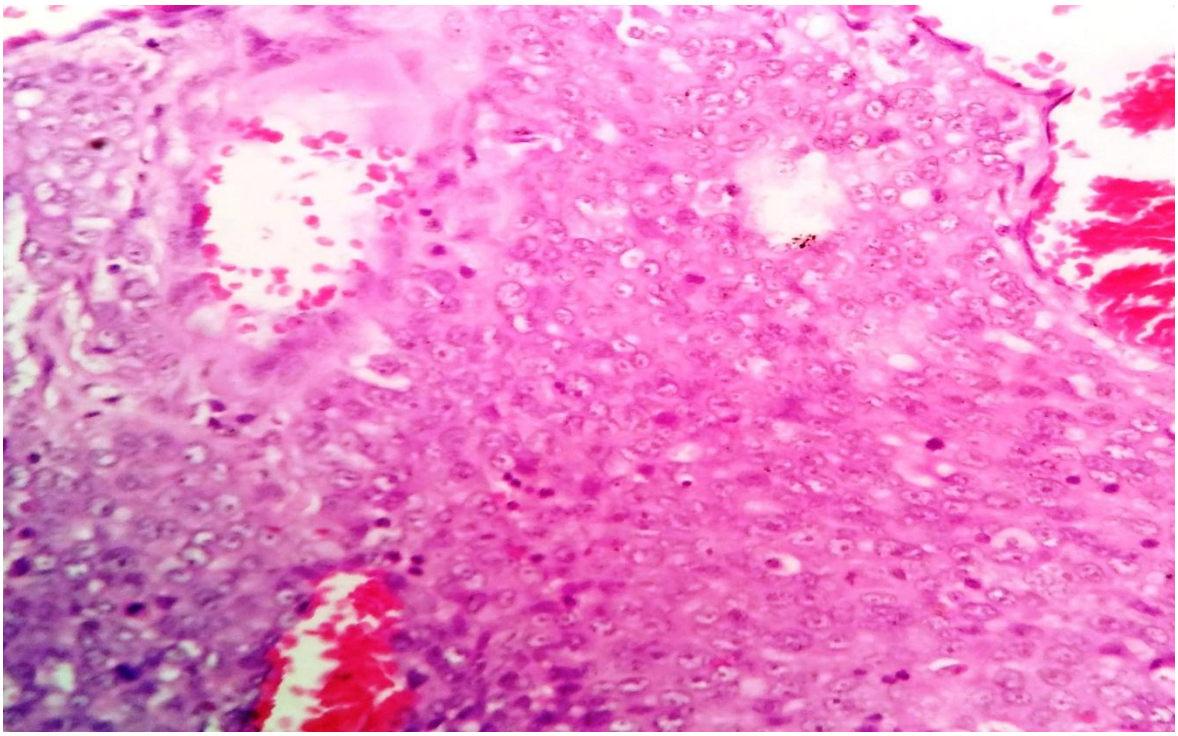


Bx 5127/15- malignant epithelial in sheets with moderate nuclear pleomorhism (400 X, H&E)

INVASIVE DUCTAL CARCINOMA NOS- GRADE III

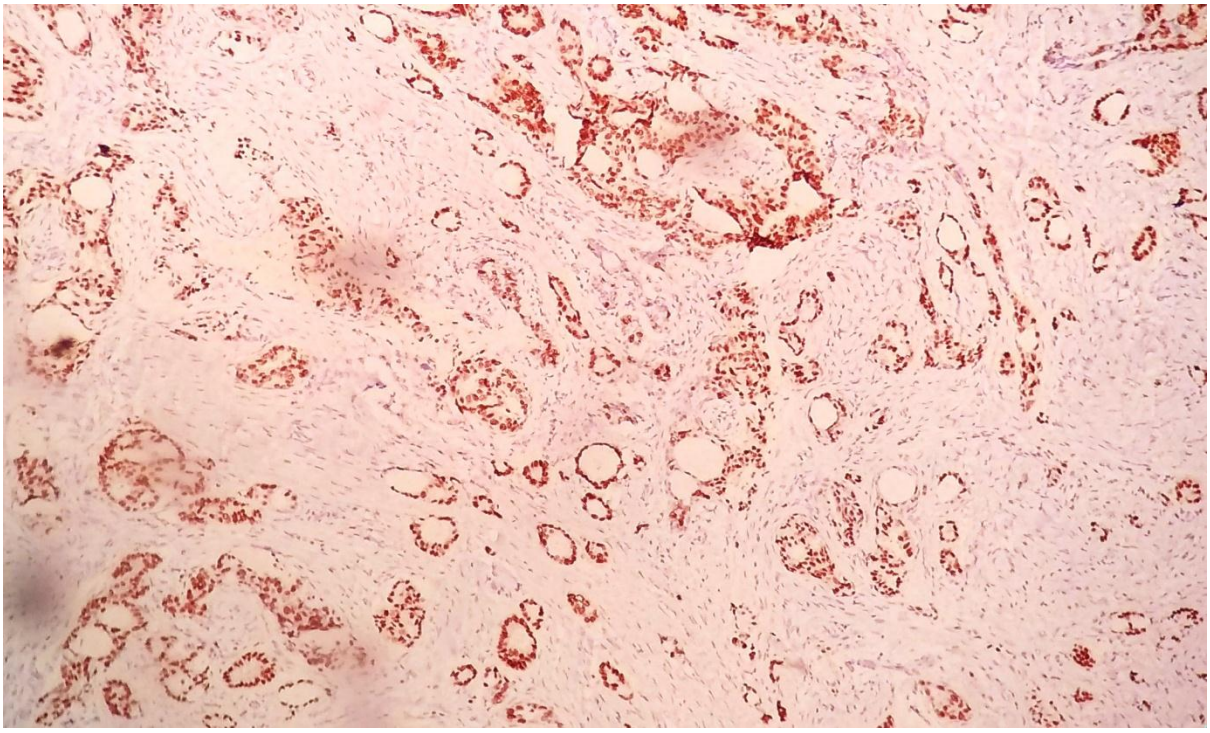


BX-11928/14- Malignant epithelial cells in sheets with interspersed blood vessels (40 X, H & E)

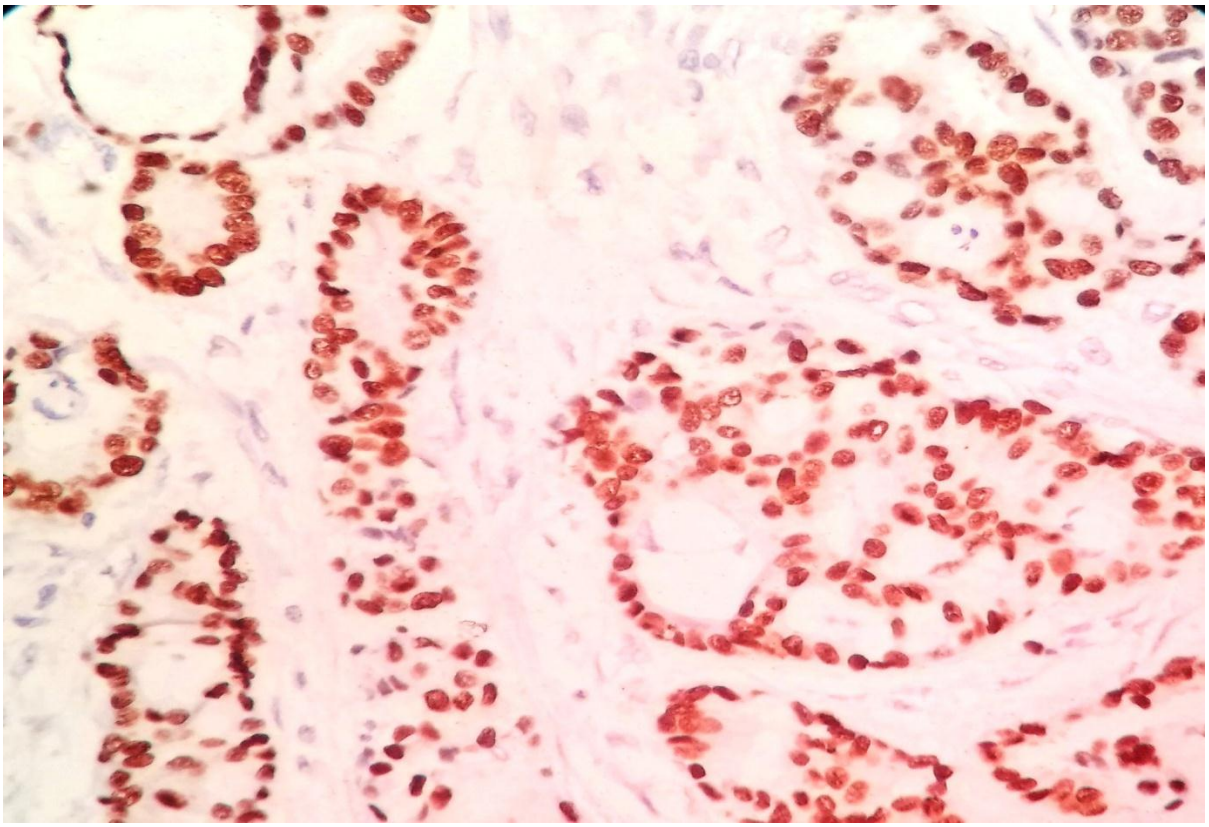


BX-11928/14- Malignant duct epithelial cells with marked pleomorphism and increased mitosis (400X, H&E)

ER

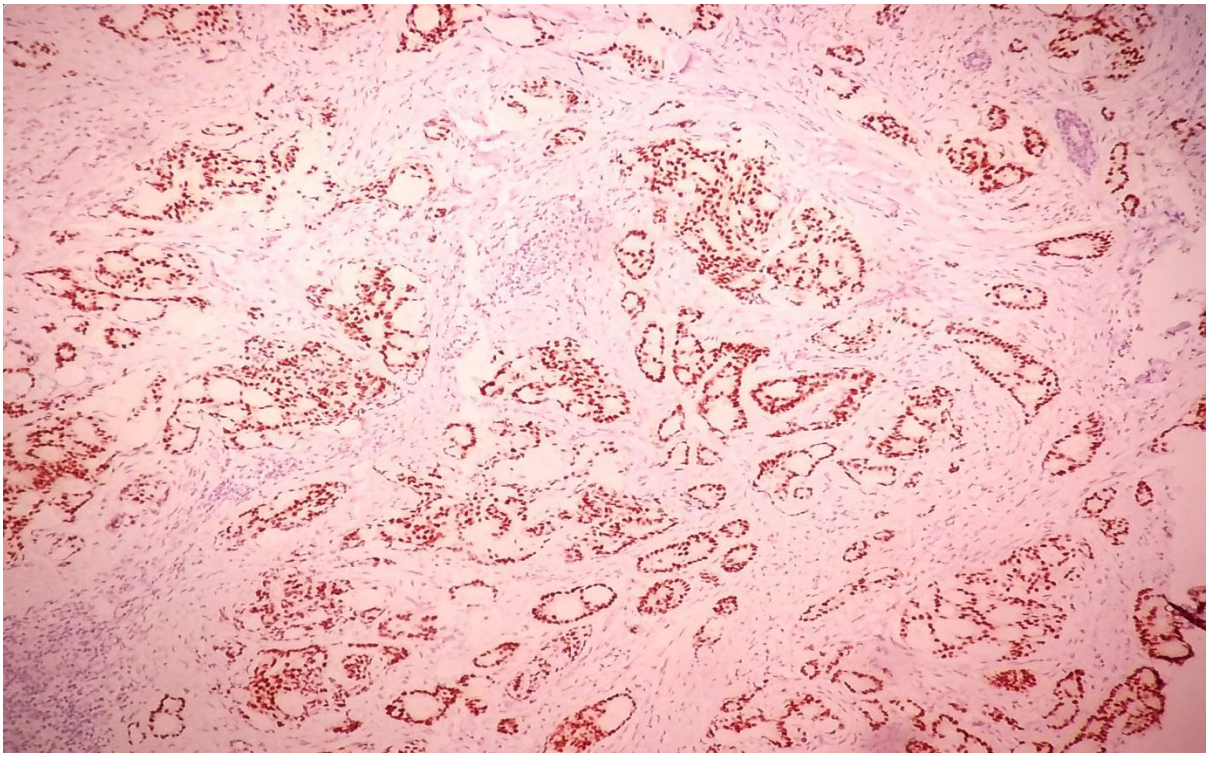


BX-4431/15 – INVASIVE DUCTAL CARCINOMA – NOS Positive for ER (100 X)

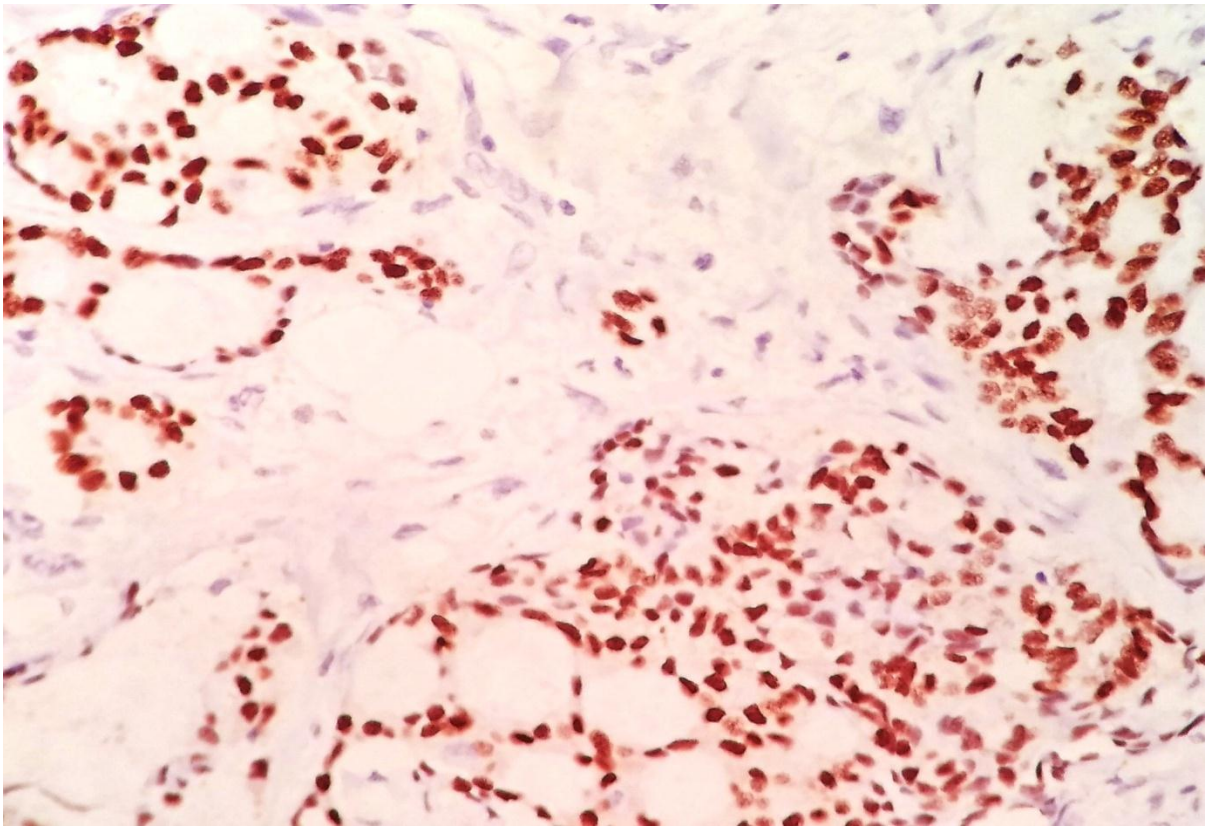


BX-4431/15 – INVASIVE DUCTAL CARCINOMA – NOS Positive for ER (400 X)

PR

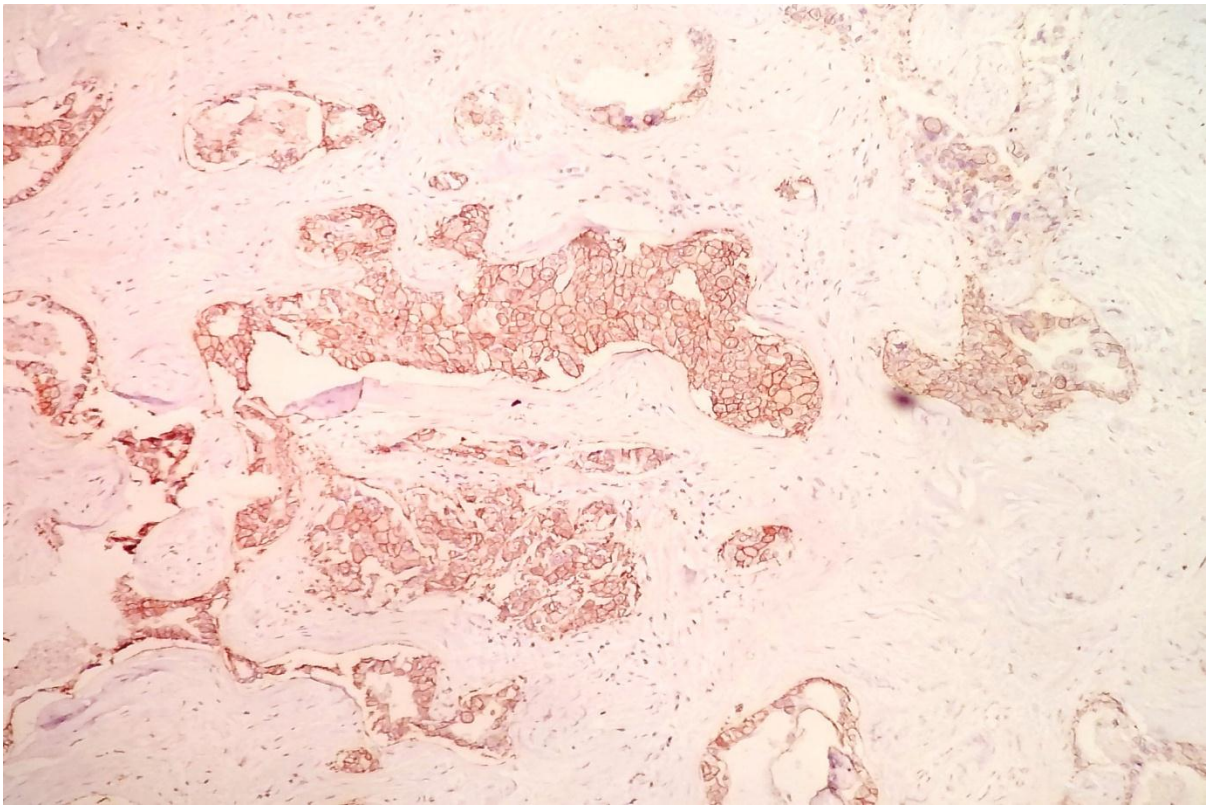


BX-4431/15 – INVASIVE DUCTAL CARCINOMA – NOS Positive for PR (100 X)

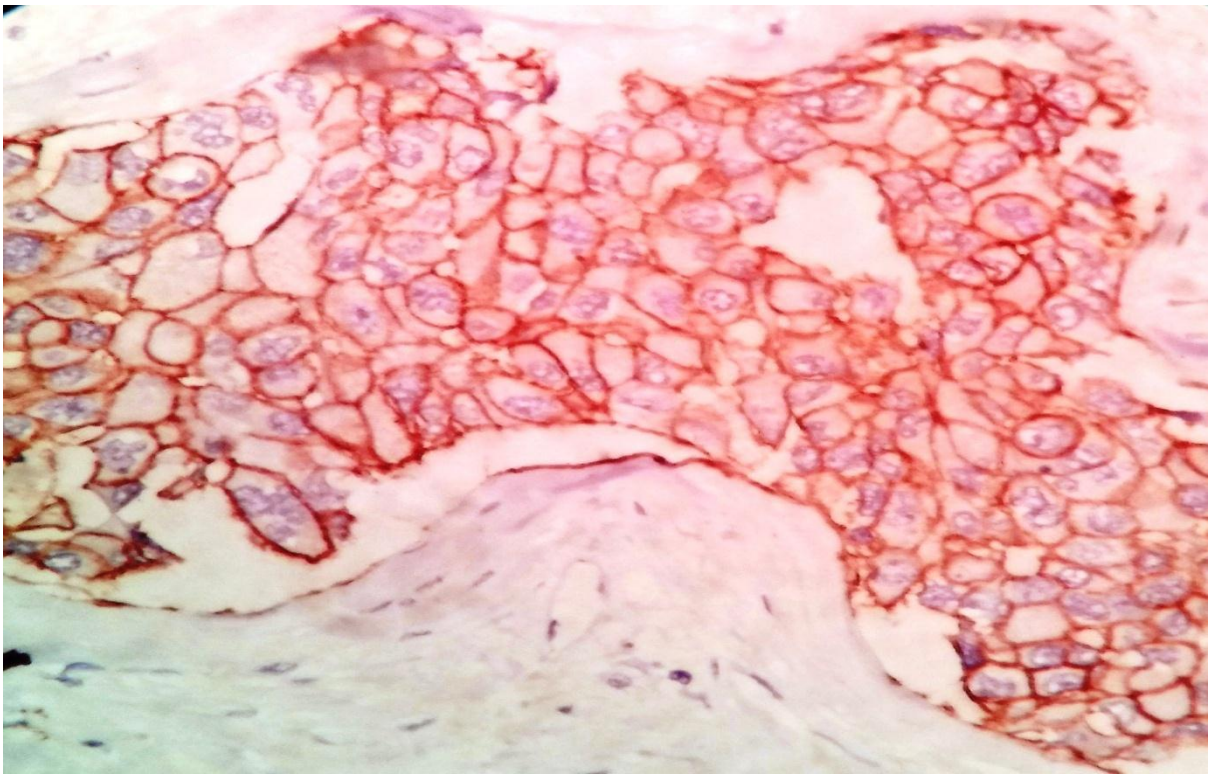


BX-4431/15 – INVASIVE DUCTAL CARCINOMA – NOS Positive for PR (400 X)

HER2 Neu

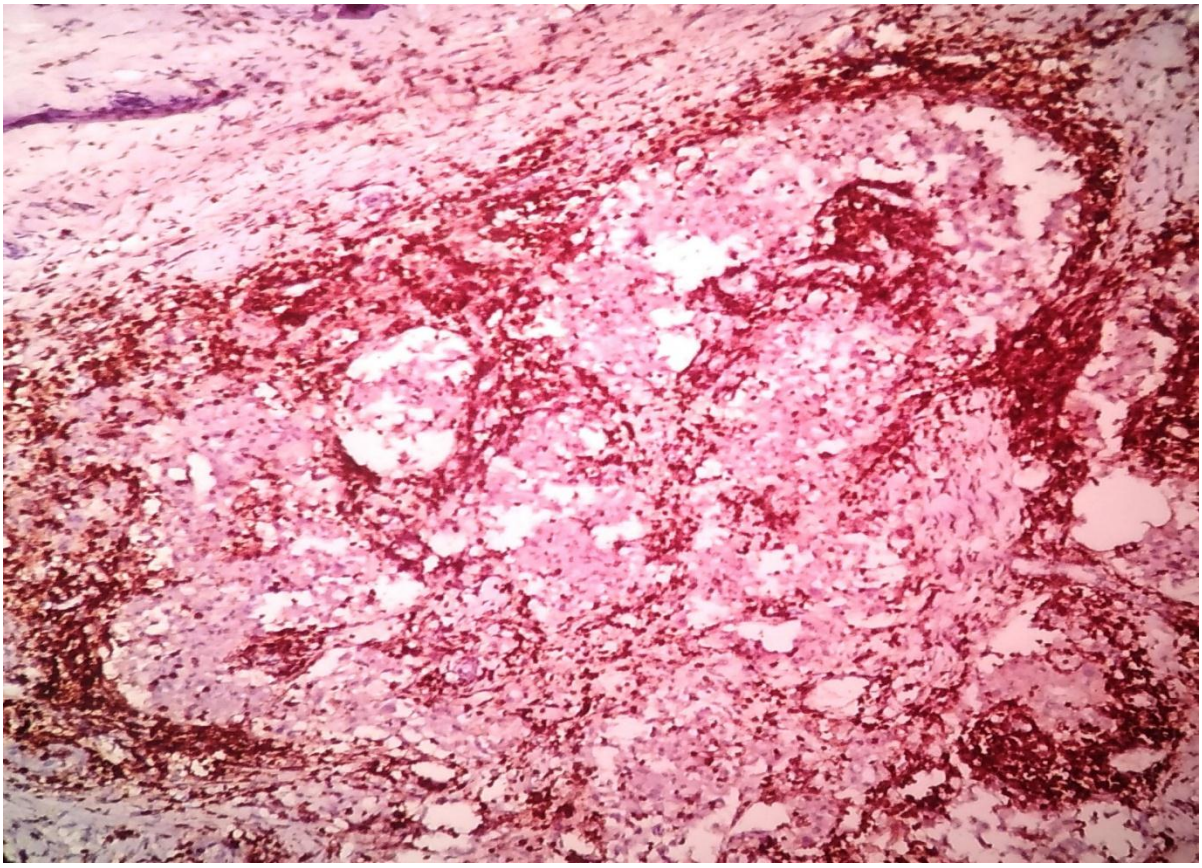


BX 11413/14- Invasive ductal carcinoma NOS- positive for HER2 neu (100X)

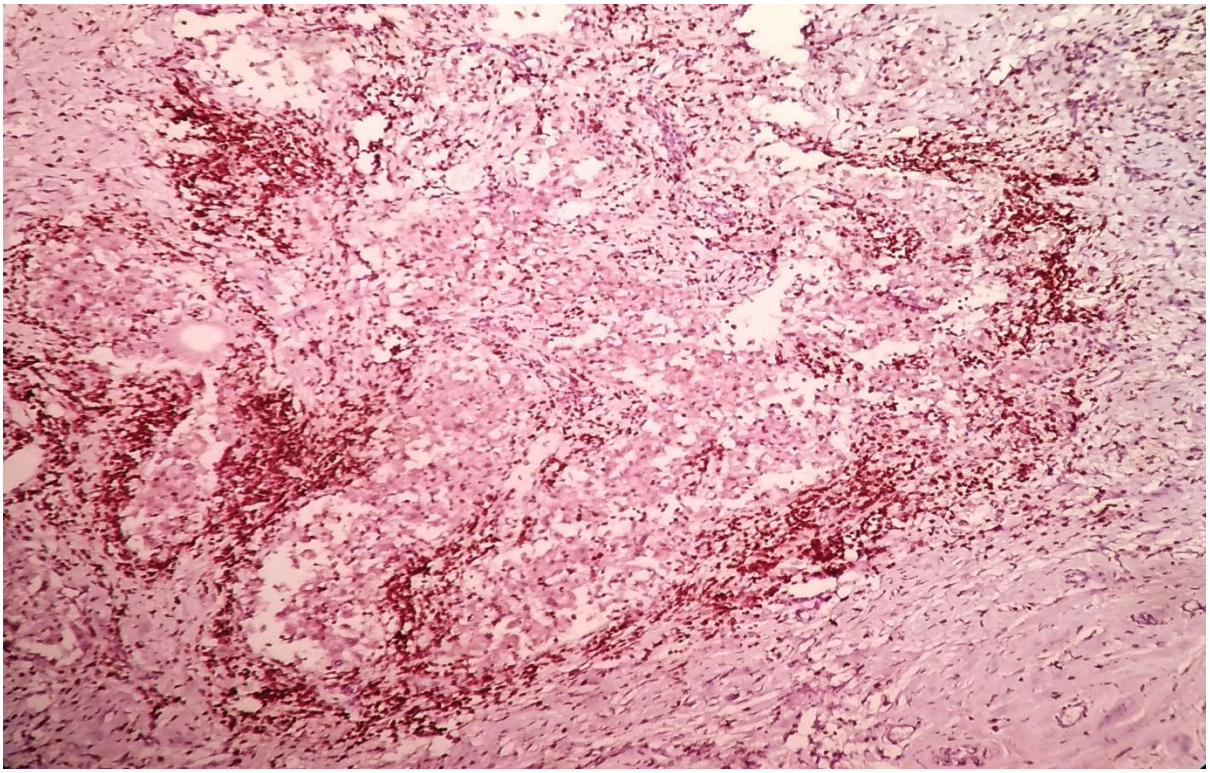


BX 11413/14- Invasive ductal carcinoma NOS- positive for HER2 neu (400X)

TUMOR INFILTRATING LYMPHOCYTES (50- 90%)

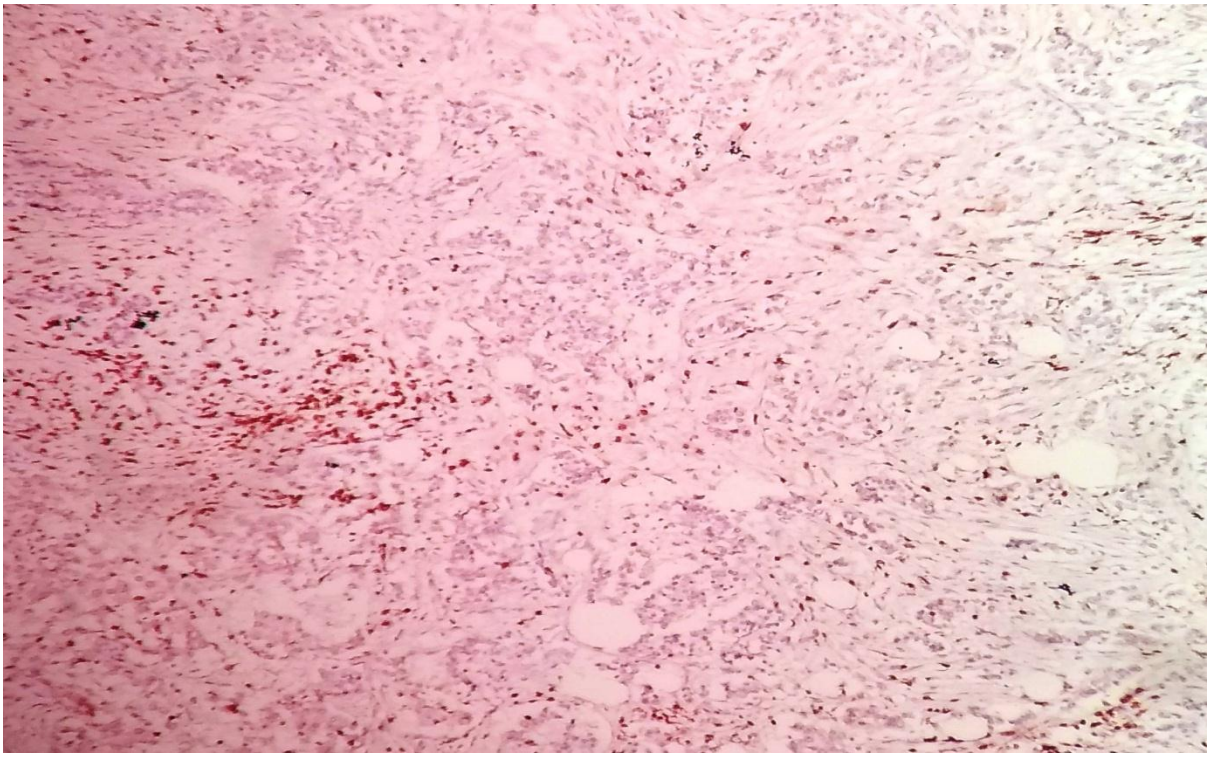


BX 5473/13- CD 45 expressed in 50-90 % of stromal cells(100 X)

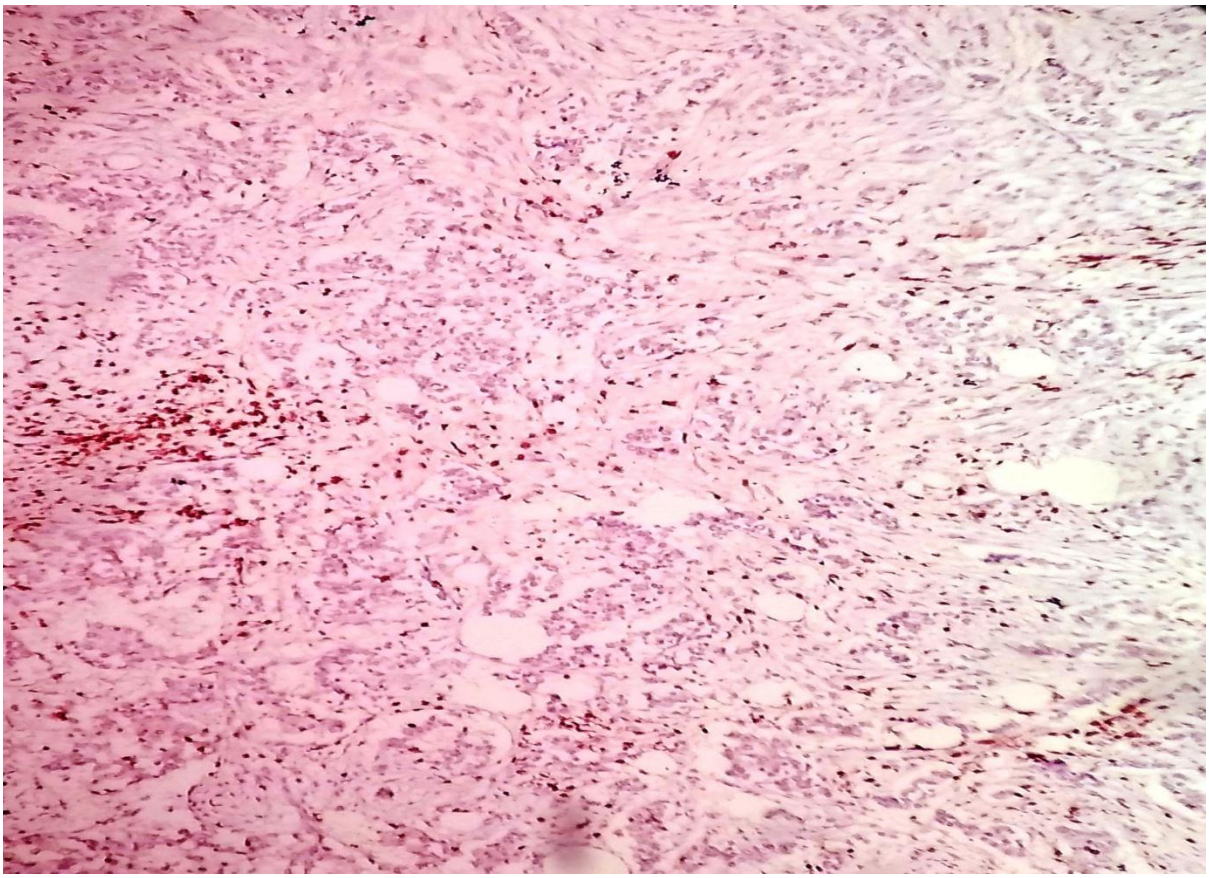


BX 5473/13- CD 3 expressed in 50-90 % of stromal cells(100 X)

TUMOR INFILTRATING LYMPHOCYTES (20-40 %)

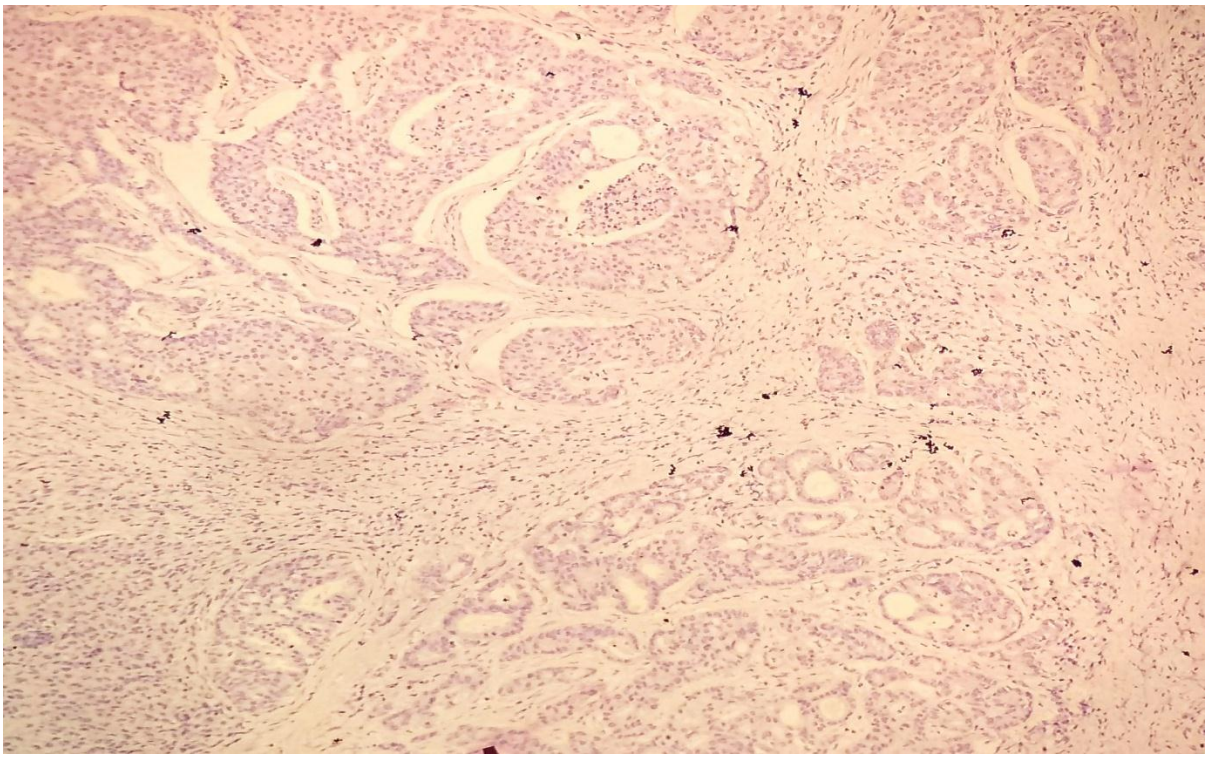


BX 3309/14 - CD 45 expressed in 20- 40% of stromal cells.(100 X)

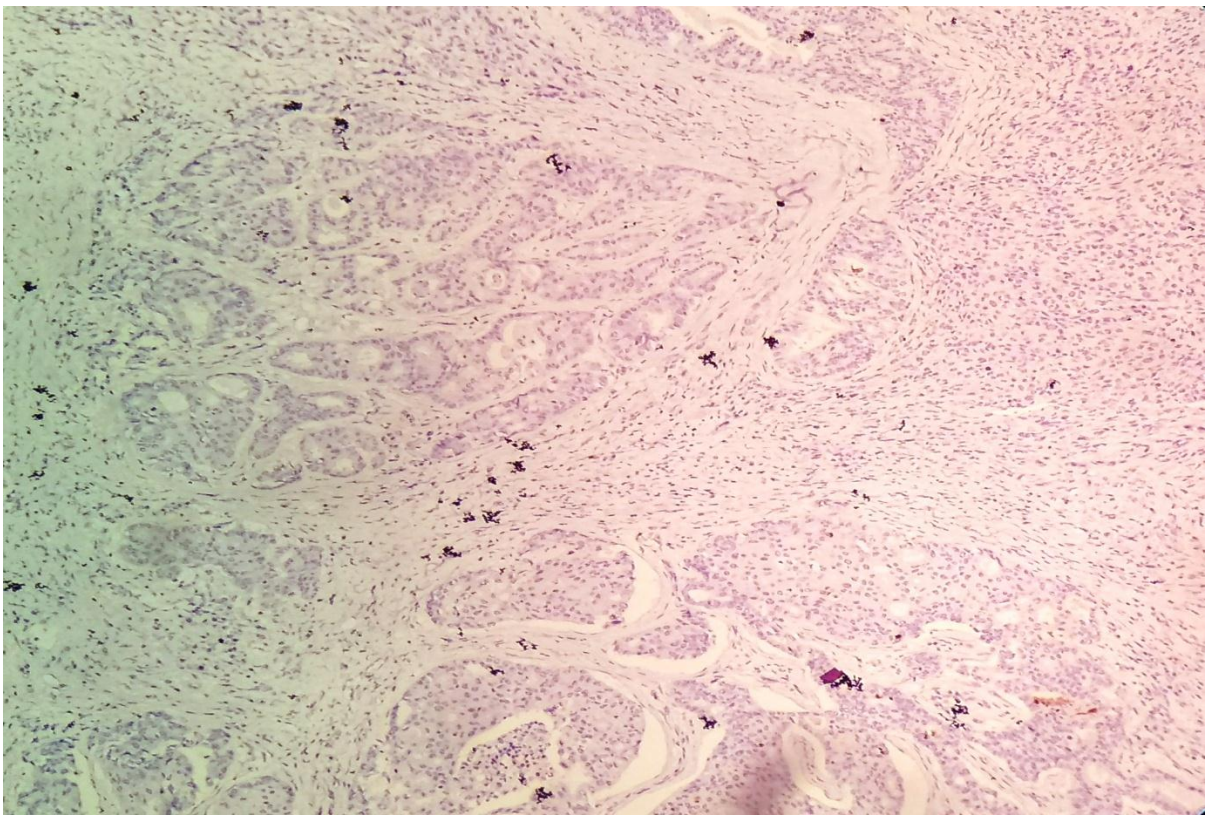


BX 3309/ 14- CD 3 expressed in 20-40 % of stromal cells (100 X)

TUMOR INFILTRATING LYMPHOCYTES (0-10 %)



BX 10598/14- CD 45 expressed in 0-10 % of stromal cells.(100 X)



BX 10598 /14- CD 3 expressed in 0-10% of stromal cells(100 X)

ANNEXURES

ANNEXURE I

PROFORMA

Case number : Name :

HPE number : Age :

IP number : Sex :

Clinical diagnosis: Menstrual status :

Risk factors if any :

Side of breast : Right/Left

Specimen : Simple Mastectomy /Modified radical Mastectomy / Radical Mastectomy/ Toilet Mastectomy /others

GROSS

Specimen size :

Nipple areola : Skin :

Tumor size : Tumor margin :

Appearance :

Resected margins : Superior : Inferior :

Medial : Lateral :

Posterior :

Associated findings :

Total number of nodes dissected :

Largest node size :

MICROSCOPY

Histological subtype :

Histological score : Nuclear score : Mitotic score :

Modified Scarf Bloom Richardson GRADE :I / II / III

Skin : Free / involved

Nipple & Areola : Free/ Involved

Margins : Superior : Free / Involved

Inferior : Free / Involved

Medial : Free /Involved

Lateral : Free /Involved

Posterior : Free/Involved

Lymphatic invasion : Present /Absent

Vascular Invasion : Present /Absent

Lymphocytic infiltration : Present / Absent

Necrosis : Present /Absent

Associated breast lesions :

Total number of nodes dissected :

Number of nodes involved :

ANNEXURE II

TNM classification of carcinomas of the breast:

T	-	Primary tumor
TX	-	Primary tumor cannot be assessed
T0	-	No evidence of primary tumor
Tis	-	Carcinoma in situ
Tis (DCIS)	-	Ductal carcinoma in situ
Tis (LCIS)	-	Lobular carcinoma in situ
Tis (Paget)	-	Paget disease of the nipple with no tumor.

(Note- Carcinomas in the breast parenchyma associated with Paget disease are categorized based on the size and characteristics of the parenchymal disease)

T1	-	Tumor ≤ 20 mm in greatest dimension
T1mi	-	Tumor ≤ 1 mm in greatest dimension
T1a	-	Tumor > 1 mm but ≤ 5 mm in greatest dimension
T1b	-	Tumor > 5 mm but ≤ 10 mm in greatest dimension
T1c	-	Tumor > 10 mm but ≤ 20 mm in greatest dimension
T2	-	Tumor > 20 mm but ≤ 50 mm in greatest dimension
T3	-	Tumor > 50 mm in greatest dimension
T4	-	Tumor of any size with direct extension to the chest wall and/or to the skin (ulceration or skin nodules)
T4a	-	Extension to chest wall, not including only pectoralis muscle adherence/invasion
T4b	-	Ulceration and/or ipsilateral satellite nodules and/or edema (including peau d'orange) of the skin
T4c	-	Both T4a and T4b
T4d	-	Inflammatory carcinoma

ANNEXURE III

NOTTINGHAM MODIFICATION OF SCARF BLOOM

RICHARDSON GRADING SYSTEM

TUBULE FORMATION	**SCORE**
Tubular formations in >75% of the tumor	1
Tubular formations in 10–75% of the tumor	2
Tubular formations in <10% of the tumor	3
NUCLEAR PLEOMORPHISM	**SCORE**
Nuclei with minimal variation in size and shape	1
Nuclei with Moderate variation in size and shape	2
Nuclei with marked variation in size and shape	3
MITOTIC RATE	**SCORE**
<10 mitosis / 10 high power field	1
10 – 20 mitosis / 10 high power field	2
>20 mitosis / 10 high power field	3

GRADE	SCORE
Grade I	3,4,5
Grade II	6,7
Grade III	8,9

ANNEXURE IV

ALLRED SCORE FOR ESTROGEN AND PROGESTERONE RECEPTORS

PROPOTIONAL SCORE(PS):

0- No staining
1- Staining of < 1 % of tumor cells
2- Staining between 1% and 10 % of tumor cells
3- Staining between 1/10 and 1/3 of tumor cells
4- Staining between 1/3 and 2/3 of tumor cells
5- Staining of > 2/3 of tumor cells

INTENSITY SCORE (IS):

0- No staining
1- Average weak intensity
2- Average moderate intensity
3- Average strong intensity

Allred score (range, 0 to 8) = PS+ IS

Possible Allred scores are
1) 0 = negative,
2) 2-8 = diffusely & strongly positive tumor.

Scoring for HER2neu

Score	Staining pattern	classification
0	No reactivity / membranous reactivity in < 10 % of cells	Negative
1+	Faint membranous reactivity in > 10 % of cells: reactive only in part of the membrane	Negative
2+	Weak to moderate/ complete or basolateral membranous reactivity in > 10 % of tumor cells	Equivocal(to be confirmed with FISH)
3+	Moderate to strong / basolateral membranous reactivity in > 10 % of tumor cells	Positive

INFORMATION SHEET

- We are conducting a study on breast cancer among patients attending Government General Hospital, Chennai and for that your specimen may be valuable to us.
- The purpose of this study is to aid in prognostic value of Tumor Infiltrating Lymphocytes with the help of Immunohistochemical markers.
- We are selecting certain cases and if your specimen is found eligible, we may be using your specimen to perform extra tests and special studies which in any way do not affect your final report or management.
- The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.
- Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.
- The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of investigator Signature of participant

Date:

INFORMED CONSENT FORM

Title of the study : **Evaluation of Tumor Infiltrating Lymphocytes In Breast Cancer**

Name of the Participant :

Name of the Principal (Co-Investigator) :

Name of the Institution : Madras Medical College

Name and address of the sponsor / agency (ies) (if any) :

Documentation of the informed consent

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in **" Evaluation of Tumor Infiltrating Lymphocytes In Breast Cancer"**

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study in which the resected Mastectomy specimens will be subjected to immunohistochemistry and histopathological examination.
4. I have been explained about my rights and responsibilities by the investigator. I have the right to withdraw from the study at any time.
5. I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.
6. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.
7. I have understood that my identity will be kept confidential if my data are publicly presented
8. I have had my questions answered to my satisfaction.
9. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

For adult participants:

Name and signature / thumb impression of the participant (or legal representative if participant
incompetent)

Name _____ Signature_____ Date_____

Name and Signature of impartial witness (required for illiterate patients):

Name _____ Signature_____ Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____ Signature_____ Date_____

BIBLIOGRAPHY

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MASTER CHART

Bx.no	AGE	SIDE	QUADRANT	SIZE	CHEMOTHERAPY	DIAGNOSIS	GRADE	NODES	ER	PR	her2neu	CD 45(%)	CD 3 (%)	TILs
122/15	41-50	left	subareolar	2-5CM		IDC with focal medullary differentiation	II	0-3	neg	neg	pos	50-90	50-90	50-90
177/15	41-50	left	all	>5CM		IDC-NST	III	0-3	neg	neg	neg	20-40	20-40	20-40
353/15	61-70	left	inner	1-2CM	given	IDC-NST	II	0-3	pos	pos	neg	20-40	20-40	20-40
412/15	51-60	left	central	2-5CM		IDC-NST	III	0-3	neg	neg	neg	50-90	50-90	50-90
656/15	41-50	left	central	>5CM		IDC-NST	II	0-3	neg	neg	neg	20-40	20-40	20-40
703/15	51-60	left	central	2-5CM	given	IDC-NST	II		pos	pos	neg	20-40	20-40	20-40
1097/15	31-40	right	central	2-5CM	given	IDC-NST	II	0-3	neg	neg	pos	0-10	0-10	0-10
1586/15	51-60	left	all	1-2CM		Metaplastic ca		0-3	neg	neg	neg	0-10	0-10	0-10
1590/15	51-60	right	central	1-2CM	given	IDC-NST	II	0-3	pos	pos	neg	0-10	0-10	0-10
1689/15	41-50	left	inner	2-5CM		IDC-NST	II	0-3	neg	neg	neg	20-40	20-40	20-40
1968/15	51-60	right	all	>5CM		IDC with focal apocrine change	III		neg	neg	neg	20-40	20-40	20-40
2392/15	41-50	right	outer	2-5CM		IDC-NST	I	0-3	pos	pos	neg	50-90	50-90	50-90
2809/15	51-60	left	outer	1-2CM		IDC-NST	II		neg	neg	neg	0-10	0-10	0-10
2840/15	41-50	left	outer	1-2CM		IDC-NST	II		neg	neg	pos	0-10	0-10	0-10
2849/15	41-50	left	outer	2-5CM	given	IDC-NST	I		pos	pos	pos	0-10	0-10	0-10
2858/15	51-60	left	inner	>5CM		IDC with papillary features	I		pos	neg	neg	50-90	50-90	50-90
3115/15	51-60	right	outer	2-5CM		IDC-NST	I		neg	neg	neg	20-40	20-40	20-40
3270/15	61-70	right	central	2-5CM	given	IDC-NST	II		pos	pos	pos	20-40	20-40	20-40
3405/15	41-50	right	outer	2-5CM		IDC-NST	I	>10	pos	pos	neg	20-40	20-40	20-40
3678/15	41-50	left	central	1-2CM	given	IDC-NST	I	>3-9	pos	pos	pos	50-90	50-90	50-90
3803/15	41-50	left	central	2-5CM		IDC-NST	II	>3-9	neg	neg	neg	20-40	20-40	20-40
3854/15	31-40	left	all	>5CM	Given	IDC-NST	II	0-3	neg	neg	pos	20-40	20-40	20-40
3893/15	51-60	right	central	1-2CM		IDC-NST	I	>10	neg	neg	neg	20-40	20-40	20-40
3927/15	41-50	left	central	2-5CM		IDC-NST	II	0-3	pos	pos	pos	20-40	20-40	20-40
4230/15	61-70	left	central	2-5CM	given	IDC-NST	II	0-3	neg	neg	pos	20-40	20-40	20-40
4339/15	41-50	left	inner	1-2CM		IDC-NST	I	> 3-9	pos	neg	neg	50-90	50-90	50-90
4431/15	61-70	left	outer	2-5CM		IDC-NST	I	0-3	pos	pos	neg	20-40	20-40	20-40

4556/15	61-70	right	central	2-5CM		IDC-NST	I	>3-9	pos	neg	neg	50-90	50-90	50-90
5127/15	31-40	left	inner	1-2CM		IDC-NST	II	0-3	neg	neg	neg	50-90	50-90	50-90
9413/15	31-40	left	outer	2-5CM	given	IDC-NST	II		pos	neg	neg	20-40	20-40	20-40
10014/15	51-60	left	central	>5CM	given	IDC-NST	III	0-3	neg	neg	neg	50-90	50-90	50-90
10105/15	51-60	right	outer	1-2CM		IDC-NST	II	0-3	pos	pos	neg	20-40	20-40	20-40
10160/15	61-70	left	inner	>5CM		IDC-NST	II	0-3	neg	neg	neg	20-40	20-40	20-40
10175/15	31-40	left	outer	2-5CM		IDC-NST	I	0-3	pos	pos	pos	50-90	50-90	50-90
10273/15	41-50	left	outer	1-2CM		IDC-NST	I	> 3-9	pos	pos	neg	20-40	20-40	20-40
10524/15	61-70	left	outer	2-5CM		IDC-NST	II	0-3	neg	neg	neg	50-90	50-90	50-90
10748/15	51-60	right	inner	1-2CM	given	IDC-NST	II	0-3	pos	pos	neg	0-10	0-10	0-10
10855/15	31-40	left	central	>5CM		IDC-NST	I	0-3	neg	neg	neg	20-40	20-40	20-40
10937/15	41-50	left	all	2-5CM		IDC-NST	I	0-3	pos	pos	pos	50-90	50-90	50-90
10955/15	41-50	right	central	1-2CM		IDC-NST	I	0-3	neg	neg	pos	20-40	20-40	20-40
11040/15	61-70	right	central	2-5CM		IDC-NST	II	>3- 9	neg	neg	neg	20-40	20-40	20-40
11994/14	41-50	right	central	>5CM	given	IDC-NST	II	0-3	pos	pos	neg	50-90	50-90	50-90
11928/14	51-60	right	central	2-5CM	given	IDC-NST	III		neg	neg	pos	20-40	20-40	20-40
11889/14	31-40	right	outer	2-5CM	given	IDC-NST	II	0-3	pos	neg	neg	20-40	20-40	20-40
11516/14	31-40	right	outer	>5CM		IDC-NST	II	0-3	neg	neg	neg	20-40	20-40	20-40
11413/14	41-50	right	central	1-2CM	given	IDC-NST	II	0-3	neg	neg	pos	50-90	50-90	50-90
11334/14	31-40	left	central	>5CM	given	IDC-NST	II	0-3	pos	pos	neg	50-90	50-90	50-90
11283/14	51-60	left	all	2-5CM	given	IDC-NST	II	>10	pos	neg	neg	20-40	20-40	20-40
11191/14	41-50	right	inner	1-2CM		IDC-NST	II	0-3	pos	pos	pos	50-90	50-90	50-90
11184/14	61-70	left	central	>5CM		IDC-NST	III	0-3	neg	neg	neg	20-40	20-40	20-40
11141/14	41-50	right	outer	2-5CM		IDC-NST	II	0-3	neg	neg	pos	20-40	20-40	20-40
11094/14	41-50	left	central	>5CM		IDC-NST	II	>3-9	pos	pos	pos	50-90	50-90	50-90
10939/14	41-50	right	inner	>5CM		IDC-NST	II	>10	pos	pos	pos	20-40	20-40	20-40
10850/14	51-60	right	all	1-2CM		IDC-NST	II	0-3	neg	neg	neg	20-40	20-40	20-40
10598/14	61-70	left	inner	2-5CM	given	IDC-NST	II	0-3	pos	pos	neg	0-10	0-10	0-10
10449/14	41-50	left	inner	1-2CM		IDC-NST	III	0-3	neg	neg	neg	50-90	50-90	50-90

10374/14	31-40	right	outer	2-5CM		IDC-NST	II	> 3 - 9	pos	pos	pos	0-10	0-10	0-10
10118/14	51-60	right	central	1-2CM	given	IDC-NST	II	> 3-9	pos	pos	pos	20-40	20-40	20-40
10005/14	51-60	left	outer	2-5CM		IDC-NST	II	0-3	neg	neg	neg	50-90	50-90	50-90
9897/14	41-50	left	central	2-5CM	given	IDC-NST	II	> 3 -9	neg	neg	pos	20-40	20-40	20-40
9823/14	41-50	left	outer	>5CM		IDC-NST	II		neg	neg	neg	0-10	0-10	0-10
9714/14	41-50	right	all	>5CM	given	IDC-NST	I		pos	pos	pos	20-40	20-40	20-40
9711/14	41-50	left	inner	2-5CM	given	IDC-NST	II		neg	neg	pos	20-40	20-40	20-40
9667/14	31-40	left	central	1-2CM	given	IDC-NST	II	0-3	pos	neg	neg	0-10	0-10	0-10
9334/14	31-40	right	outer	2-5CM		IDC-NST	II	0-3	neg	neg	neg	50-90	50-90	50-90
9275/14	51-60	left	central	>5CM	given	IDC-NST	III		pos	pos	pos	20-40	20-40	20-40
8327/14	51-60	left	outer	1-2CM	given	IDC-NST	III		pos	pos	pos	50-90	50-90	50-90
8287/14	51-60	left	outer	1-2CM		IDC-NST	II	0-3	pos	neg	neg	20-40	20-40	20-40
6903/14	41-50	left	inner	2-5CM	given	IDC-NST	II	0-3	pos	pos	neg	0-10	0-10	0-10
6906/14	41-50	right	outer	2-5CM	given	IDC-NST	II	> 3 - 9	pos	pos	neg	20-40	20-40	20-40
5206/14	51-60	left	central	>5CM	given	IDC-NST	II	0-3	pos	pos	neg	20-40	20-40	20-40
5332/14	51-60	right	central	2-5CM	given	Mucinous Carcinoma		0-3	neg	neg	neg	0-10	0-10	0-10
5353/14	41-50	left	inner	1-2CM		IDC -medullary variant			neg	neg	neg	20-40	20-40	20-40
5784/14	41-50	right	outer	2-5CM	given	IDC-NST	II	> 3 - 9	pos	pos	pos	50-90	50-90	50-90
6018/14	51-60	left	central	1-2CM	given	IDC-NST	II	0-3	neg	neg	pos	0-10	0-10	0-10
4195/14	31-40	right	inner	2-5CM	given	IDC-NST	II	0-3	pos	neg	neg	20-40	20-40	20-40
3309/14	51-60	left	inner	1-2CM	given	IDC-NST	II	0-3	pos	pos	neg	20-40	20-40	20-40
3384/14	31-40	right	outer	2-5CM	given	IDC-NST	I		neg	neg	pos	20-40	20-40	20-40
1228/14	61-70	right	outer	2-5CM	given	IDC-NST	II	0-3	pos	pos	pos	50-90	50-90	50-90
1793/14	71-80	left	all	1-2CM	given	IDC-NST	II	0-3	pos	neg	neg	0-10	0-10	0-10
11234/13	51-60	left	central	>5CM	given	IDC-NST	III	> 3 - 9	pos	pos	pos	20-40	20-40	20-40
11295/13	51-60	right	outer	2-5CM	given	IDC-NST	III	0-3	neg	neg	pos	50-90	50-90	50-90
11141/13	31-40	right	central	1-2CM	given	IDC-NST	II	0-3	pos	pos	neg	50-90	50-90	50-90
10512/13	31-40	left	central	1-2CM	given	IDC-NST	II		neg	neg	pos	20-40	20-40	20-40
9097/13	31-40	left	central	2-5CM	given	IDC-NST	II	0-3	pos	pos	neg	20-40	20-40	20-40

9682/13	41-50	left	central	2-5CM	given	IDC-NST	II	0-3	pos	neg	neg	50-90	50-90	50-90
9873/13	31-40	left	outer	>5CM	given	IDC-NST	III		pos	neg	neg	50-90	50-90	50-90
8540/13	31-40	left	inner	1-2CM	given	IDC-NST	II	0-3	pos	pos	neg	20-40	20-40	20-40
8593/13	61-70	right	central	2-5CM	given	IDC-NST	III	0-3	neg	neg	pos	20-40	20-40	20-40
7327/13	51-60	right	all	2-5CM	given	IDC-NST	III		pos	pos	neg	20-40	20-40	20-40
7433/13	21-30	right	central	2-5CM	given	Medullary ca			neg	neg	pos	50-90	50-90	50-90
8084/13	41-50	left	all	1-2CM	given	IDC-NST	II	0-3	pos	neg	neg	20-40	20-40	20-40
6515/13	51-60	right	central	2-5CM	given	IDC-NST	II	0-3	pos	pos	neg	20-40	20-40	20-40
6636/13	31-40	left	inner	1-2CM	given	IDC-NST	I	0-3	neg	neg	pos	0-10	0-10	0-10
5391/13	51-60	right	central	2-5CM	given	IDC-NST	II	0-3	pos	pos	neg	20-40	20-40	20-40
5473/13	41-50	right	central	1-2CM		IDC-NST	III	0-3	neg	neg	neg	50-90	50-90	50-90
5584/13	31-40	left	all	>5CM		IDC-NST	II	0-3	pos	pos	pos	20-40	20-40	20-40
270/13	41-50	left	central	2-5CM		IDC-NST	III	> 3 -9	neg	neg	neg	20-40	20-40	20-40
158/1`3	31-40	left	central	2-5CM		IDC-NST	I		neg	neg	neg	50-90	50-90	50-90
1128/13	51-60	left	central	>5CM		IDC-NST	II		neg	neg	neg	50-90	50-90	50-90

Neg- Negative **Pos- Positive** **IDC- NST – Infiltrating Ductal Carcinoma- No Special Type**